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GB05/918

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JHC/P041113GB1

09MAR04 EB79486-2 D02973

2. Patent application number

0405267.6

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3. Full name, address and postcode of the or of each applicant (underline all surnames)

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09 MAR 2004

Patents ADP number (if you know it)

7516081001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

COMPOUNDS

5. Name of your agent (if you have one)

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Description 85

Claim(s) 5

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COMPOUNDS**BACKGROUND**

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The present disclosure relates to pharmaceutically useful products obtainable from organoboronic acids. The disclosure also relates to the use of members of the aforesaid class of products, to their formulation, their preparation, their synthetic intermediates and to other subject matter.

10 The disclosure further relates to pharmaceutical formulations containing the described products.

Boropeptide Serine Protease Inhibitors

15 Shenvi (EP-A-145441 and US 4499082) disclosed that peptides containing an α -aminoboronic acid with a neutral side chain were effective inhibitors of elastase and has been followed by numerous patent publications relating to boropeptide inhibitors of serine proteases.

20 In describing inhibitors or substrates of proteases, P1, P2, P3, etc. designate substrate or inhibitor residues which are amino-terminal to the scissile peptide bond, and S1, S2, S3, etc., designate the corresponding subsites of the cognate protease in accordance with: Schechter, I. and Berger, A. On the Size of the Active Site in Proteases, *Biochem.Biophys.Res.Comm.*, 27:157-162, 1967. In thrombin, the S1 binding site or "specificity pocket" is a well defined slit in the enzyme, whilst the S2 and S3 binding subsites (also respectively called the proximal and distal hydrophobic pockets) are hydrophobic and interact strongly with, respectively, Pro and (R)-Phe, amongst others.

25

Aminoboronate or peptidoboronate inhibitors or substrates of serine proteases are described in:

- US 4935493
- EP 341661
- 30 • WO 94/25049
- WO 95/09859
- WO 96/12499
- WO 96/20689
- Lee S-L et al, *Biochemistry* 36:13180-13186, 1997
- 35 • Dominguez C et al, *Bioorg. Med. Chem. Lett.* 7:79-84, 1997
- EP 471651
- WO 94/20526
- WO 95/20603
- WO97/05161

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- US 4450105
- US 5106948
- US 5169841
- WO 96/25427
- 5 ▪ US 5288707
- WO 96/20698
- WO 01/02424

10 The amino acid sequence (R)-Phe-Pro-Arg, imitating amino acid sequences of fibrinogen, was at one time considered the best sequence for thrombin inhibitors. This sequence formed tight-binding inhibitors of thrombin, e.g. Ac-(R)-Phe-Pro-boroArg (DUP 714), having K_i values in the picomolar range (Kettner et al, *J. Biol. Chem.* 265: 18289-18297, 1990; EP-A-293,881).

15 The replacement of the P2 Pro residue of borotripeptide thrombin inhibitors by an N-substituted glycine is described in Fevig J M et al *Bioorg. Med. Chem.* 8: 301-306 and Rupin A et al *Thromb. Haemost.* 78(4):1221-1227, 1997. See also US 5,585,360 (de Nanteuil et al).

20 Matteson D S *Chem. Rev.* 89: 1535-1551, 1989 reviews the use of α -halo boronic esters as intermediates for the synthesis of *inter alia* amino boronic acids and their derivatives. Matteson describes the use of pinacol boronic esters in non-chiral synthesis and the use of pinanediol boronic esters for chiral control, including in the synthesis of amino and amido boronate esters.

25 Adams et al, US Patent No (1998), US Patent No 6066730 (2000), US Patent No 6083903 (2000) and equivalent, and US Patent No 6297217 (2001) describe peptide boronic ester and acid compounds useful as proteasome inhibitors. These documents also describe the use of boronic ester and acid compounds to reduce the rate of muscle protein degradation, to reduce the activity of NF- κ B in a cell, to reduce the rate of degradation of p53 protein in a cell, to inhibit cyclin degradation in a cell, to inhibit the growth of a cancer cell, to inhibit antigen presentation in a cell, to inhibit NF- κ B dependent cell adhesion, and to inhibit HIV replication. Brand et al, WO 98/35691, teaches that 30 proteasome inhibitors, including boronic acid compounds, are useful for treating infarcts such as occur during stroke or myocardial infarction. Elliott et al, WO 99/15183, teaches that proteasome inhibitors are useful for treating inflammatory and autoimmune diseases.

35 Unfortunately, organoboronic acids can be relatively difficult to obtain in analytically pure form. Thus, alkylboronic acids and their boroxines are often air-sensitive. Korček et al, *J. Chem. Soc. Perkin Trans.* 2:242, 1972, teaches that butylboronic acid is readily oxidized by air to generate 1-butanol and boric acid.

It is known that derivatisation of boronic acids as cyclic esters provides oxidation resistance. For example, Martichonok V et al *J. Am. Chem. Soc.* 118: 950-958, 1996 state that diethanolamine derivatisation provides protection against possible boronic acid oxidation. US Patent No 5,681,978 (Mattheson DS et al) teaches that 1,2-diols and 1,3 diols, for example pinacol, form stable cyclic boronic esters that are not easily oxidised.

Wu et al, *J. Pharm. Sci.*, 89:758-765, 2000, discuss the stability of the compound N-(2-pyrazine) carbonyl-phenylalanine-leucine boronic acid (LDP-341, also known as bortezomib), an anti-cancer agent. It is described how "during an effort to formulate [LDP-341] for parenteral administration, 10 the compound showed erratic stability behaviour". The degradation pathways were investigated and it was concluded that the degradation was oxidative, the initial oxidation being attributed to peroxides or molecular oxygen and its radicals.

WO 02/059131 discloses boronic acid products which are described as stable. In particular, these 15 products are certain boropeptides and/or boropeptidomimetics in which the boronic acid group has been derivatised with a sugar.

Neutral P1 Residue Boropeptide Thrombin Inhibitors

20 Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose lipophilic thrombin inhibitors having a neutral (uncharged) C-terminal (P1) side chain, for example an alkoxyalkyl side chain.

25 The Claeson et al and Kakkar et al patent families disclose boronate esters containing the amino acid sequence D-Phe-Pro-BoroMpg [(R)-Phe-Pro-BoroMpg], which are highly specific inhibitors of thrombin. Of these compounds may be mentioned in particular Cbz-(R)-Phe-Pro-BoroMpg-OPinacol (also known as TRI 50b). The corresponding free boronic acid is known as TRI 50c. For further information relating to TRI 50b and related compounds, the reader is referred to the following documents:

30

- Elgendy S et al., in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, 340:173-178, 1993.
- Claeson G et al, *Biochem J.* 290:309-312, 1993
- Tapparelli C et al, *J Biol Chem.* 268:4734-4741, 1993
- Claeson G, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, 340:83-91, 1993

35

- Phillip et al, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, 340:67-77, 1993
- Tapparelli C et al, *Trends Pharmacol. Sci.* 14:366-376, 1993
- Claeson G, *Blood Coagulation and Fibrinolysis* 5:411-436, 1994

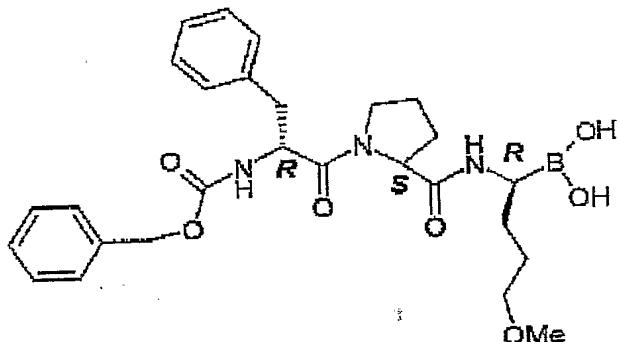
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- Elgendi et al, *Tetrahedron* 50:3803-3812, 1994
- Deadman J et al, *J. Enzyme Inhibition* 9:29-41, 1995
- Deadman J et al, *J. Medicinal Chemistry* 38:1511-1522, 1995.

5 TRI 50b is considered to be a prodrug for TRI 50c, which is the active principal *in vivo*. The tripeptide sequence of TRI 50c has three chiral centres. The Phe residue is considered to be of (R)-configuration and the Pro residue of natural (S)-configuration, at least in compounds with commercially useful inhibitor activity; the Mpg residue is believed to be of (R)-configuration in isomers with commercially useful inhibitor activity. Thus, the active, or most active, TRI 50c isomers with commercially useful inhibitor activity. Thus, the active, or most active, TRI 50c stereoisomer is considered to be of R,S,R configuration and may be represented as:

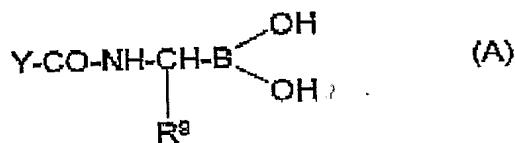
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Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH

15 PCT/GB03/03897, and also USSN 10/659,178 and EP-A-1396270, disclose pharmaceutically acceptable base addition salts of boronic acids which have a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked through a peptide linkage to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites. In a first embodiment, there is disclosed a pharmaceutically acceptable base addition salt of a boronic acid of, for example, formula (A):

20



wherein

Y comprises a hydrophobic moiety which, together with the aminoboronic acid residue $-\text{NHCH}(\text{R}^9)-\text{B}(\text{OH})_2$, has affinity for the substrate binding site of thrombin; and

R⁹ is a straight chain alkyl group interrupted by one or more ether linkages (e.g. 1 or 2) and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R⁹ is -(CH₂)_m-W where m is 2, 3, 4 or 5 (e.g. 4) and W is -OH or halogen (F, Cl, Br or I). R⁹ is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

5

Also disclosed are oral formulations of such salts.

The salts are described as being of relative stability to hydrolysis and deboronation.

10 PCT/GB03/03887, and also USSN 10/659,179 and EP-A-1396269, disclose salts of a pharmaceutically acceptable multivalent (at least divalent) metal and an organoboronic acid drug. Such salts are described as having an improved level of stability which cannot be explained or predicted on the basis of known chemistry, and as being indicated to have unexpectedly high and consistent oral bioavailability not susceptible of explanation on the basis of known mechanisms. The oral
15 formulations of such salts are therefore also disclosed.

One particular class of salts comprises those wherein the organoboronic acid comprises a boropeptide or boropeptidomimetic. Boropeptide drugs which may beneficially be prepared as salts include without limitation those of the formula X-(aa)_n-B(OH)₂, where X is H or an amino-protecting
20 group, n is 2, 3 or 4, (especially 2 or 3) and each aa is independently a hydrophobic amino acid, whether natural or unnatural. In one class of multivalent metal salts, the organoboronic acid is of formula (A) above.

25 PCT/GB03/03883, and also USSN 10/658,971 and EP-A-1400245, disclose and claim *inter alia* parenteral pharmaceutical formulations that include a pharmaceutically acceptable base addition salt of a boronic acid of, for example, formula (A) above. Such salts are described as having an improved level of stability which cannot be explained or predicted on the basis of known chemistry.

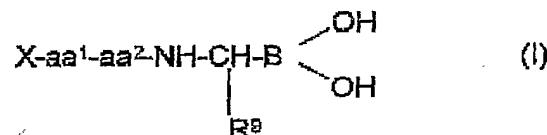
30 In one aspect, the present disclosure relates, at least in embodiments, to organoboronic acid compounds designed to have an extended *in vivo* half life. In another aspect, the disclosure relates, at least in embodiments, to thrombin-inhibitory products which, at least in broad terms, maintain or improve the potency or specificity, or both, of such products whose active principle is TRI 50c.

35 In further aspects, disclosed compounds may, at least in embodiments, broadly maintain or enhance bioavailability. Other properties of disclosed compounds which may lend the compounds pharmaceutical usefulness may include storage stability or ease of formulation, for example.

BRIEF SUMMARY OF THE DISCLOSURE

The disclosure concerns novel organoboronic acid thrombin inhibitors and the salts and prodrugs thereof (which prodrugs may themselves be in the form of salts).

5 In one aspect, the disclosure relates to boronic acids which have a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites. It also relates to salts and prodrugs of such acids. In a first embodiment, there is disclosed a boronic acid of, for example, formula (I):

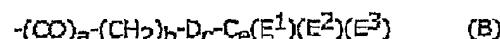
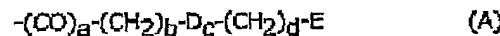


10

wherein

X is H (to form NH_2) or an amino-protecting group;

15 aa¹ is an amino acid residue having a side chain selected from formula (A) and (B):



20

wherein

a is 0 or 1;

e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 5 or, as the case may be, (b+e) is from 1 to 5;

25

c is 0 or 1;

D is O or S;

E is a saturated or unsaturated cyclic hydrocarbyl group which normally contains up to 14 members; and

30 E¹, E² and E³ are each independently selected from the group consisting of 5-6 membered saturated or unsaturated hydrocarbyl rings, or one of E¹, E² and E³ is hydrogen and the other two are a said hydrocarbyl ring,

E, E¹, E² and E³ optionally being halogenated when saturated and mandatorily being halogenated when unsaturated, a particular halogen being fluorine;

35

aa² is a residue of an amino carboxylic acid which binds to the thrombin S2 subsite; and

R⁹ is a straight chain alkyl group interrupted by one or more ether linkages (e.g. 1 or 2) and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R⁹ is -(CH₂)_m-W

5 where m is 2, 3, 4 or 5 (e.g. 4) and W is -OH or halogen (F, Cl, Br or I). R⁹ is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

aa¹ may be a fluorinated Phe or Dpa residue.

10 The disclosure includes not only the acids as such but also their acid addition salts, base addition salts, prodrugs and prodrug salts.

15 Disclosed as certain examples are hydrophobic boronic acid inhibitors of thrombin, whether in the form of the free acid or, for example, their base addition salts. All the boronic acid thrombin inhibitors described herein may be in the form of prodrugs, or acid or base addition salts of prodrugs.

20 As suitable prodrugs may be mentioned esters, e.g. with a residue of an alkanol, e.g. a C₁-C₄ alkanol such as methanol or ethanol, for example. It may be an ester of a diol.

25 The identity of the diol is not critical. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting carbon atoms in a chain or ring. One class of diols comprises hydrocarbons substituted by exactly two hydroxy groups. One such diol is pinacol and another is pinanediol and a third is diethanolamine; there may also be mentioned neopentylglycol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

30 The prodrug may be a sugar derivative as described in WO 02/059131 (see above). Thus, the boronate group may be esterified with a sugar such as a monosaccharide or disaccharide, for example. The sugar may be a reduced sugar, e.g. mannitol or sorbitol; it may be any individual sugar or class of sugars taught in WO 02/059131. The boronic acid, sugar (or other diol) and water may be combined and then lyophilised, for example as taught in WO 02/059131.

35 The disclosed thrombin inhibitors may contain hydrophobic amino acids, and this class of amino acids includes those whose side chain is hydrocarbyl, hydrocarbyl containing an in-chain oxygen and/or linked to the remainder of the molecule by an in-chain oxygen or heteroaryl, or any of the

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aforsaid groups when substituted by hydroxy, halogen or trifluoromethyl. Representative hydrophobic side chains include alkyl, alkoxyalkyl, either of the aforesaid when substituted by at least one aryl or heteroaryl, aryl, heteroaryl, aryl substituted by at least one alkyl and heteroaryl substituted by at least one alkyl. Proline and other imino acids which are ring-substituted by nothing or by one of the moieties listed in the previous sentence are also hydrophobic.

5

Some hydrophobic side chains contain from 1 to 20 carbon atoms, e.g. non-cyclic moieties having 1, 2, 3 or 4 carbon atoms. Side chains comprising a cyclic group typically but not necessarily contain from 5 to 13 ring members and in many cases are phenyl or alkyl substituted by one or two phenyls.

10

Included are inhibitors which contain hydrophobic non-peptide moieties, which are typically based on moieties which may form a side chain of a hydrophobic amino acid, as described above.

15

Hydrophobic compounds may contain, for example, one amino group and/or one acid group (e.g. -COOH, -B(OH)₂). Generally, they do not contain multiple polar groups of any one type.

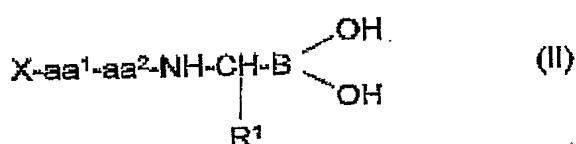
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The disclosure comprises hydrophobic boronic acid inhibitors of thrombin, and therefore includes peptide boronic acids which have a partition coefficient between 1-n-octanol and water expressed as log P of greater than 1.0 at physiological pH and 25°C. Some peptide boronic acids useful in the invention have a partition coefficient of at least 1.5. A class of hydrophobic peptide boronic acids useful in the invention has a partition coefficient of no more than 5.

25

Some sub-classes of hydrophobic organoboronic acids are those described by Formulae (I) and (III) below, under the heading "Detailed Description of Several Examples".

Also disclosed as another embodiment is a peptide boronic acid of formula (II):



where:

30

X is a moiety bonded to the N-terminal amino group and may be H to form NH₂. The identity of X is not critical but may be a particular X moiety described above. In one example there may be mentioned benzylloxycarbonyl.

R¹ is a group of the formula $-(\text{CH}_2)_m-\text{W}$, where m is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, Br or I).

aa¹ is a residue of an amino acid having a C₁-C₅ alkyl substituted by one or two moieties selected from fluorophenyl, cyclohexyl and fluorocyclohexyl, the fluorophenyl and fluorocyclohexyl moieties often being substituted by fluorine at the 4-position and optionally at one or more additional positions.

As previously described, the acid may be in the form of a salt (acid or base addition salt), prodrug or salt of a prodrug.

aa² is a residue of an imino acid having from 4 to 6 ring members. Alternatively, aa² is a residue of Gly N-substituted by a C₃-C₁₃ hydrocarbyl group, e.g. a C₃-C₈ hydrocarbyl group comprising a C₃-C₆ hydrocarbyl ring; the hydrocarbyl group may be saturated, for example exemplary N-substituents 15 are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. As a hydrocarbyl group containing one or more unsaturated bonds may be mentioned phenyl and methyl or ethyl substituted by phenyl, e.g. 2-phenylethyl, as well as β,β -dialkylphenylethyl. Other aa² moieties are subsequently described.

There is a debate in the literature as to whether boronates in aqueous solution form the 'trigonal' B(OH)₂ or 'tetrahedral' B(OH)₃⁻ boron species, but NMR evidence seems to indicate that at a pH below the first pKa of the boronic acid the main boron species is the neutral B(OH)₂. In the duodenum the pH is likely to be between 6 and 7, so the trigonal species is likely to be predominant here. In any event, the symbol $-\text{B}(\text{OH})_2$ includes tetrahedral as well as trigonal boron species, and throughout this specification symbols indicating trigonal boron species embrace also tetrahedral species. The symbol may further include boronic groups in anhydride form.

The boronic acids and their derivatives (salts, prodrugs, prodrug salts) may be in the form of solvates, particularly hydrates.

30 The base addition salts may comprise, or consist essentially of, acid salts in which the boronic acid is singly deprotonated. The disclosure therefore includes products having a metal/boronate stoichiometry consistent with the boronate groups in the product predominantly (more than 50 mol %) carrying a single negative charge.

35 The boronic acids and their derivatives may be in isolated form. The compounds may have a purity, e.g. as determined by the method of Example 9, of at least about 90%, e.g. of greater than or equal

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to about 95%. In the case of pharmaceutical formulations, such compound forms may be combined with pharmaceutically acceptable diluents, excipients or carriers.

5 Pharmaceutical formulations of the compounds are also provided herein. There are provided parenteral formulations, e.g. comprising the compounds in the solid phase, for example particulate salts or sugar esters for reconstitution as aqueous solutions prior to administration by injection or infusion. Such reconstituted solutions are also included in the disclosure. There are also provided oral preparations, e.g. tablets, capsules, powders or granules.

10 According to a further aspect of the present disclosure, there is provided a method of treatment of a condition where anti-thrombotic activity is required which method comprises administration of a therapeutically effective amount of a boronic acid disclosed herein, or a salt, prodrug or prodrug salt thereof, to a person suffering from, or at risk of suffering from, such a condition.

15 The disclosure includes methods for preparing the described compounds.

The base addition salts described herein include products obtainable by (having the characteristics of a product obtained by) reaction of the boronic acid with a strong base and the term "salt" herein is to be understood accordingly. The term "salt" in relation to the disclosed products, therefore, does not necessarily imply that the products contain discrete cations and anions and is to be understood as embracing products which are obtainable using a reaction of a boronic acid and a base. The disclosure embraces products which, to a greater or lesser extent, are in the form of a coordination compound. The disclosure thus provides also products obtainable by (having the characteristics of a product obtained by) reaction of a disclosed boronic acid with a strong base as well as the therapeutic, including prophylactic, use of such products.

30 The present disclosure is not limited as to the method of preparation of the base addition salts, provided that they contain a boronate species derived from a disclosed boronic acid and a counterion. Such boronate species may be boronate anions in any equilibrium form thereof. The term "equilibrium form" refers to differing forms of the same compounds which may be represented in an equilibrium equation (e.g. boronic acid in equilibrium with a boronic anhydride and in equilibrium with different boronate ions). Boronates in the solid phase may form anhydrides and the disclosed boronate salts when in the solid phase may comprise boronate anhydrides, as a boronic equilibrium species. It is not required that the salts be prepared by reaction of a base containing the counterion and the boronic acid (I). Further, the disclosure includes salt products which might be regarded as indirectly prepared by such an acid/base reaction as well as salts obtainable by (having the characteristics of products obtained by) such indirect preparation. As examples of possibly indirect preparation may be mentioned processes in which, after initial recovery of the salt, it is purified

and/or treated to modify its physicochemical properties, for example to modify solid form or hydrate form, or both.

In some embodiments, the cations of the base addition salts are monovalent.

5

In some embodiments the disclosed compounds (e.g. free boronic acids or salts) comprise anhydride species; in others they are essentially free of anhydride species.

10 10

Further aspects and embodiments of the disclosure are set forth in the following description and claims.

15

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

20

Data indicate that the stability (resistance to deboronation) of organoboronic acids may be increased by providing them in the form of salts, e.g. metal salts. In single experiments, the ammonium salt of TRI 50c appeared to decompose on drying to yield ammonia, whilst the choline salt demonstrated rapid decomposition to a deboronated impurity. Although experiments have not been conducted to reproduce these unrepeatable observations, there is provided a sub-class of the presently disclosed salts in which the ammonium and choline salts are excluded.

25

DETAILED DESCRIPTION OF SEVERAL EXAMPLES

Glossary

The following terms and abbreviations are used in this specification:

30

The expression "acid salt" as applied to a base addition salt of a boronic acid refers to salts of which a single -OH group of the trigonally-represented acid group -B(OH)₂ is deprotonated. Thus salts wherein the boronate group carries a single negative charge and may be represented as -B(OH)(O⁻) or as [-B(OH)₂]⁻ are acid salts. The expression encompasses salts of a cation having a valency n wherein the molar ratio of boronic acid to cation is approximately n to 1. In practical terms, the observed stoichiometry is unlikely to be exactly n:1 but will be consistent with a notional n:1 stoichiometry. For example, the observed mass of the cation might vary from the calculated mass for a n:1 stoichiometry by no more than about 10%, e.g. no more than about 7.5%; in some cases an observed mass of a cation might vary from the calculated mass by no more than about 1%. Calculated masses are suitably based on the trigonal form of the boronate. (At an atomic level, a

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salt stoichiometrically consistent with being an acid salt might contain boronates in a mix of protonation states, whose average approximates to single deprotonation and such "mixed" salts are included in the term "acid salt"). Examples of acid salts are monosodium salts and hemicalcium salts.

5

α -Aminoboronic acid or Boro(aa) refers to an amino acid in which the CO_2 group has been replaced by BO_2 .

The term "amino-group protecting moiety" refers to any group used to derivatise an amino group, 10 especially an N-terminal amino group of a peptide or amino acid. Such groups include, without limitation, alkyl, acyl, alkoxy carbonyl, aminocarbonyl, and sulfonyl moieties. However, the term "amino-group protecting moiety" is not intended to be limited to those particular protecting groups that are commonly employed in organic synthesis, nor is it intended to be limited to groups that are readily cleavable.

15

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a 20 reasonable benefit/risk ratio.

The expression "thrombin inhibitor" refers to a product which, within the scope of sound pharmacological judgement, is potentially or actually pharmaceutically useful as an inhibitor of thrombin, and includes reference to substance which comprises a pharmaceutically active species 25 and is described, promoted or authorised as a thrombin inhibitor. Such thrombin inhibitors may be selective, that is they are regarded, within the scope of sound pharmacological judgement, as selective towards thrombin in contrast to other proteases; the term "selective thrombin inhibitor" includes reference to substance which comprises a pharmaceutically active species and is described, promoted or authorised as a selective thrombin inhibitor.

30

The term "heteroaryl" refers to a ring system which has at least one (e.g. 1, 2 or 3) in-ring heteroatoms and has a conjugated in-ring double bond system. The term "heteroatom" includes oxygen, sulfur and nitrogen, of which sulfur is sometimes less preferred.

35 "Natural amino acid" means an L-amino acid (or residue thereof) selected from the following group of neutral (hydrophobic or polar), positively charged and negatively charged amino acids:

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Hydrophobic amino acids

A = Ala = alanine

V = Val = valine

I = Ile = isoleucine

5

L = Leu = leucine

M = Met = methionine

F = Phe = phenylalanine

P = Pro = proline

W = Trp = tryptophan

10

Polar (neutral or uncharged) amino acids

N = Asn = asparagine

C = Cys = cysteine

Q = Gln = glutamine

15

G = Gly = glycine

S = Ser = serine

T = Thr = threonine

Y = Tyr = tyrosine

20

Positively charged (basic) amino acids

R = Arg = arginine

H = His = histidine

K = Lys = lysine

25

Negatively charged amino acids

D = Asp = aspartic acid

E = Glu = glutamic acid.

30

Acid addition salt = a salt which is prepared from addition of a pharmaceutically acceptable acid to a compound comprising a basic group, e.g. an amino, amidino or guanadino group

ACN = acetonitrile

Amino acid = α -amino acid

Base addition salt = a salt which is prepared from addition of an inorganic base or an organic base to a free acid (in this case the boronic acid).

35

Cbz = benzoyloxycarbonyl

Cha = cyclohexylalanine (a hydrophobic unnatural amino acid)

Charged (as applied to drugs or fragments of drug molecules, e.g. amino acid residues) = carrying a charge at physiological pH, as in the case of an amino, amidino or carboxy group

Dcha = dicyclohexylalanine (a hydrophobic unnatural amino acid)

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Dpa = diphenylalanine (a hydrophobic unnatural amino acid)

Drug = a pharmaceutically useful substance, whether the active in vivo principle or a prodrug

i.v. = intravenous

Mpg = 3-methoxypropylglycine (a hydrophobic unnatural amino acid)

5 Multivalent = valency of at least two, for example two or three

Neutral (as applied to drugs or fragments of drug molecules, e.g. amino acid residues) = uncharged
= not carrying a charge at physiological pH

Pinac = Pinacol = 2,3-dimethyl-2,3-butanediol

Pinanediol = 2,3-pinane diol = 2,6,6-trimethylbicyclo [3.1.1] heptane-2,3-diol

10 Pip = pipocolinic acid

Room temperature = 25°C ± 2°C

s.c. = subcutaneous

Strong base = a base having a sufficiently high pKb to react with a boronic acid. Suitably such bases have a pKb of 7 or more, e.g. 7.5 or more, for example about 8 or more

15 THF = tetrahydrofuran

Thr = thrombin

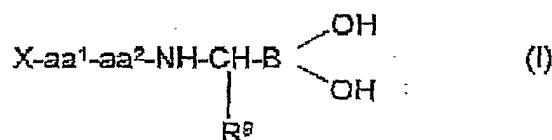
TRI 50F = the analogue of TRI 50c in which the Phe residue is 4-fluorinated

Novel Products - The Compounds

20

The disclosure relates to boronic acids and their salts, prodrugs and prodrug salts. It relates to the pharmaceutical use of these compounds.

25 The boronic acids of the disclosure comprise boronic acids which have a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked to a hydrophobic moiety capable of binding to the thrombin S2 subsite, which is linked in turn to a moiety which is capable of binding to the thrombin S3 subsite and comprises a fluorinated unsaturated ring, for example a 6-membered ring which is 4-fluorinated, or a fluorinated or unfluorinated saturated ring. The substituent on the amino group, if any, is generally an optionally substituted hydrocarbyl group, particularly an alkyl group or substituted alkyl group. The alkyl group may be halogenated, particularly fluorinated. The disclosure includes acids of formula formula (I):



wherein

35

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X is H (to form NH₂) or an amino-protecting group;

aa¹ is an amino acid residue having a side chain selected from formula (A) and (B):

5 -(CO)_a-(CH₂)_b-D_c-(CH₂)_d-E (A)

- (CO)_a-(CH₂)_b-D_c-C_e(E¹)(E²)(E³) (B)

wherein

a is 0 or 1;

10 e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 5 or, as the case may be, (b+e) is from 1 to 5;

c is 0 or 1;

D is O or S;

15 E is a saturated or unsaturated cyclic hydrocarbyl group which normally contains up to 14 members; and

E¹, E² and E³ are each independently selected from the group consisting of 5-6 membered saturated or unsaturated hydrocarbyl rings, or one of E¹, E² and E³ is hydrogen and the other two are a said hydrocarbyl ring,

20

E, E¹, E² and E³ optionally being halogenated when saturated and mandatorily being halogenated when unsaturated, a particular halogen being fluorine;

aa² is a residue of an amino acid which binds to the thrombin S2 subsite; and

25

R⁹ is a straight chain alkyl group interrupted by one or more ether linkages and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R⁹ is -(CH₂)_m-W where m is from 2, 3, 4 or 5 (e.g. 4) and W is -OH or halogen (F, Cl, Br or I). As examples of straight chain alkyl interrupted by one or more ether linkages (-O-) may be mentioned alkoxyalkyl (one interruption)

30 and alkoxyalkoxyalkyl (two interruptions). R⁹ is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

E particularly comprises a 5-6 membered ring (e.g. is 4-fluorophenyl) or an 8-14 membered fused ring system (e.g. is 4-fluoronaphthyl).

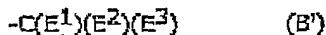
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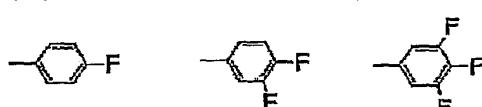
In many compounds, aa^1 has a side chain in which a is 0. If a is 1, c may be 0. Commonly, a and c are both 0. In particular examples, $(a+b+c+d)$ and $(a+b+c+e)$ are no more than 4 and are more especially 1, 2 or 3. $(a+b+c+d)$ may be 0.

5 In one class of compounds, a and c are both 0 and $(a+b+c+d)$ and $(a+b+c+e)$ are 1, 2 or 3. In particular, aa^1 has a side chain which is one of:



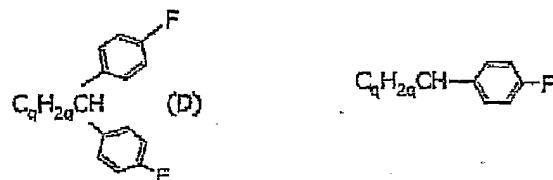
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In one class of compounds, E , E^1 , E^2 and E^3 are each independently phenyl fluorinated at at least the 4-position or optionally fluorinated cyclohexyl, the fluorinated cyclohexyl typically being fluorinated at at least the 4-position; in variants of this class one of E^1 , E^2 and E^3 is H. In this class of compounds, the number of fluorinated carbon atoms in the phenyl groups, in addition to the 4-carbon, may be 1, 2, 3 or 4. Some exemplary E , E^1 , E^2 and E^3 groups are:



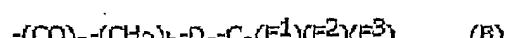
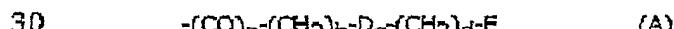
Cyclohexyl may be fluorinated as described above in relation to phenyl.

20 Particular boronic acids have aa^1 side chains selected from:



wherein wherein q is from 0 to 5, e.g. is 0, 1 or 2, especially 1 (in which latter case the side chains are fluorinated variants of Phe and Dpa). Of course, the phenyl groups may be additionally fluorinated as described above (e.g. 3,4 or 3,4,5 fluorinated).

In one class of compounds, the side chain of the P2 (S2-binding) amino acid is a moiety other than hydrogen selected from a group of formula A or B:



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wherein

a is 0 or 1;

e is 1;

5 b and d are independently 0 or an integer such that (b+d) is from 0 to 5 or, as the case may be, (b+e) is from 1 to 5;

c is 0 or 1;

D is 0 or S;

E is H, C₁-C₆ alkyl, or a saturated or unsaturated cyclic group which normally contains up to 1410 members and particularly is a 5-6 membered ring (e.g. phenyl) or an 8-14 membered fused ring system (e.g. naphthyl), which alkyl or cyclic group is optionally substituted by up to 3 groups (e.g. 1 group) independently selected from C₁-C₆ trialkylsilyl, -CN, -R¹³, -R¹²OR¹³, -R¹²COR¹³, -R¹²CO₂R¹³ and -R¹²O₂CR¹³, wherein R¹² is -(CH₂)_f- and R¹³ is -(CH₂)_gH or by a moiety whose non-hydrogen atoms consist of carbon atoms and in-ring heteroatoms and number from 5 to 14 and15 which contains a ring system (e.g. an aryl group) and optionally an alkyl and/or alkylene group, wherein f and g are each independently from 0 to 10, g particularly being at least 1 (although -OH may also be mentioned as a substituent), provided that (f+g) does not exceed 10, more particularly does not exceed 6 and most particularly is 1, 2, 3 or 4, and provided that there is only a single substituent if the substituent is a said moiety containing a ring system, or E is C₁-C₆ trialkylsilyl;20 and E¹, E² and E³ are each independently selected from -R¹⁵ and -J-R¹⁵, where J is a 5-6 membered ring and R¹⁵ is selected from C₁-C₆ trialkylsilyl, -CN, -R¹³, -R¹²OR¹³, -R¹²COR¹³, -R¹²CO₂R¹³, -R¹²O₂CR¹³, and one or two halogens (e.g. in the latter case to form a -J-R¹⁵ moiety which is dichlorophenyl), where R¹² and R¹³ are, respectively, an R¹² moiety and an R¹³ moiety as defined above (in some acids where E¹, E² and E³ contain an R¹³ group, g is 0 or 1);

25

in which moiety of Formula (A) or (B) any ring is carbocyclic or aromatic, or both, and any one or more hydrogen atoms bonded to a carbon atom is optionally replaced by halogen, especially F.

30 In certain examples, a is 0. If a is 1, c may be 0. In particular examples, (a+b+c+d) and (a+b+c+e) are no more than 4 and are more especially 1, 2 or 3. (a+b+c+d) may be 0.

Exemplary groups for E, E¹, E² and E³ include aromatic rings such as phenyl, naphthyl, pyridyl, quinolinyl and furanyl, for example; non-aromatic unsaturated rings, for example cyclohexenyl; saturated rings such as cyclohexyl, for example. E may be a fused ring system containing both 35 aromatic and non-aromatic rings, for example fluorenyl. One class of E, E¹, E² and E³ groups are aromatic (including heteroaromatic) rings, especially 6-membered aromatic rings. In some

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compounds, E¹ is H whilst E² and E³ are not H; in those compounds, examples of E² and E³ groups are phenyl (substituted or unsubstituted) and C₁-C₄ alkyl, e.g. methyl.

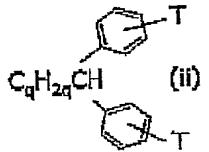
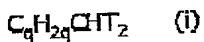
5 In one class of embodiments, E contains a substituent which is C₁-C₆ alkyl, (C₁-C₅ alkyl)carbonyl, carboxy C₁-C₅ alkyl, aryl (including heteroaryl), especially 5-membered or preferably 6-membered aryl (e.g. phenyl or pyridyl), or arylalkyl (e.g. arylmethyl or arylethyl where aryl may be heterocyclic and is preferably 6-membered).

10 In another class of embodiments, E contains a substituent which is OR¹³, wherein R¹³ can be a 6-membered ring, which may be aromatic (e.g. phenyl) or is alkyl (e.g. methyl or ethyl) substituted by such a 6-membered ring.

A class of moieties of formula A or B are those in which E is a 6-membered aromatic ring optionally substituted, particularly at the 2-position or 4-position, by $-R^{13}$ or $-OR^{13}$.

15 The disclosure includes acids in which the P3 and/or P2 side chain comprises a cyclic group in which 1 or 2 hydrogens have been replaced by halogen, e.g. F or Cl.

The disclosure includes a class of acids in which the side chains of formula (A) or (B) are of the following formulae (i), (ii) or (iii):



25 wherein q is from 0 to 5, e.g. is 0, 1 or 2, and each T is independently hydrogen, one or two halogens (e.g. F or Cl), -SiMe₃, -CN, -R¹³, -OR¹³, -COR¹³, -CO₂R¹³ or -O₂CR¹³. In some embodiments of structures (ii) and (iii), T is at the 4-position of the phenyl group(s) and is -R¹³, -OR¹³, -COR¹³, -CO₂R¹³ or -O₂CR¹³, and R¹³ is C₁-C₁₀ alkyl and more particularly C₁-C₆ alkyl. In one sub-class, T is -R¹³ or -OR¹³, for example in which f and g are each independently 0, 1, 2 or 3; in some side chains groups of this sub-class, T is -R¹²OR¹³ and R¹³ is H.

30 In one class of the moieties, the side chain is of formula (i) and each T is independently R^{13} or OR^{13} and R^{13} is C_1 - C_4 alkyl. In some of these compounds, R^{13} is branched alkyl and in others it is straight chain. In some moieties, the number of carbon atoms is from 1 to 4.

The disclosure therefore includes medicaments comprising organoboronic acids or derivatives thereof which are thrombin inhibitors, particularly selective thrombin inhibitors, having a neutral P1 (S1-binding) moiety. For more information about moieties which bind to the S3, S2 and S1 sites of thrombin, see for example Tapparelli C et al, *Trends Pharmacol. Sci.* 14: 366-376, 1993; Sanderson P et al, *Current Medicinal Chemistry*, 5: 289-304, 1998; Rewinkel J et al, *Current Pharmaceutical Design*, 5:1043-1075, 1999; and Coburn C *Exp. Opin. Ther. Patents* 11(5): 721-738, 2001. The thrombin inhibitory compounds of the disclosure are not limited to those having S3, S2 and S1 affinity groups described in the publications listed in the preceding sentence. Particular derivatives 10 are base addition salts.

The boronic acids may have a K_i for thrombin of about 100 nM or less, e.g. about 20 nM or less.

A subset of the Formula (I) acids comprises the acids of Formula (II):

15

X is a moiety bonded to the N-terminal amino group and may be H to form NH_2 . The identity of X is not critical but may be a particular X moiety described above. In one example there may be mentioned benzyloxycarbonyl.

20

In certain examples X is $\text{R}^6-(\text{CH}_2)_p-\text{C}(\text{O})-$, $\text{R}^6-(\text{CH}_2)_p-\text{S}(\text{O})_2-$, $\text{R}^6-(\text{CH}_2)_p-\text{NH}-\text{C}(\text{O})-$ or $\text{R}^6-(\text{CH}_2)_p-\text{O}-\text{C}(\text{O})-$ wherein p is 0, 1, 2, 3, 4, 5 or 6 (of which 0 and 1 are preferred) and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by one or more (e.g. 1, 2, 3, 4 or 5) halogens (e.g. F), for example at least at the 4-position, and/or by 1, 2 or 3 substituents selected from amino, nitro, hydroxy, a C₅-C₆ cyclic group, C₁-C₄ alkyl and C₁-C₄ alkyl containing, and/or linked to the 5 to 13-membered cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a C₅-C₆ cyclic group. More particularly X is $\text{R}^6-(\text{CH}_2)_p-\text{C}(\text{O})-$ or $\text{R}^6-(\text{CH}_2)_p-\text{O}-\text{C}(\text{O})-$ and p is 0, 1 or 2; in these moieties, R⁶ may be phenyl or fluorophenyl. Said 5 to 13-membered cyclic group is often aromatic or heteroaromatic, for example is a 6-membered aromatic or heteroaromatic group. The cyclic group may be saturated, particularly it may be a cycloalkyl group, e.g. cyclohexyl, optionally substituted by one or more halogens (e.g. F). When R⁶ comprises an F-substituted 6-membered ring, the ring is typically at least 4-substituted. In many cases, the group is not substituted.

25

Exemplary X groups are (2-pyrazine) carbonyl, (2-pyrazine) sulfonyl and particularly benzyloxycarbonyl or benzylmethylcarbonyl.

aa¹ is as previously described. Thus, it may have a side chain which is -CH₂-E or -C(E¹)(E²)(E³), in particular where one of E¹, E² and E³ is H. More particularly, aa¹ may be 4-F-Phe, di(4-F)-Dpa, Cha or Dcha. (4-F-Phe refers to Phe whose phenyl group is 4-fluorinated; di(4-F)-Dpa refers to Dpa both of whose phenyl groups are 4-fluorinated).

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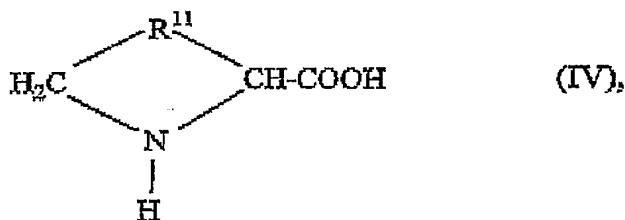
aa² is an imino acid residue having from 4 to 6 ring members. Alternatively, aa² is a residue of H₂N-CH₂-COOH N-substituted by a C₃-C₁₃ hydrocarbyl group, e.g. a C₃-C₈ hydrocarbyl group comprising a C₃-C₆ hydrocarbyl ring; the hydrocarbyl group may be saturated, for example exemplary N-substituents are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. As a hydrocarbyl group containing one or more unsaturated bonds may be mentioned phenyl and methyl or ethyl substituted by phenyl, e.g. 2-phenylethyl, as well as β,β -dialkylphenylethyl.

10 As another alternative, aa² is the β -amino acid analogue of Gly (i.e. H₂N-CH₂-CH₂-COOH) N-substituted by a C₃-C₁₃ hydrocarbyl group, e.g. a C₃-C₈ hydrocarbyl group comprising a C₃-C₆ hydrocarbyl ring; the hydrocarbyl group may be saturated, for example exemplary N-substituents are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. As a hydrocarbyl group containing one or 15 more unsaturated bonds may be mentioned phenyl and methyl or ethyl substituted by phenyl, e.g. 2-phenylethyl, as well as β,β -dialkylphenylethyl.

20 The disclosure includes a class of compounds in which aa² is a residue of a β -amino acid having a 4 to 6 membered carbocyclic ring which optionally has one carbon atom replaced by a sulfur and of which the ring-forming carbon atoms include the carbon atoms α - and β - to the carboxyl group (i.e. the β -amino acid comprises a 4 to 6 membered carbocyclic ring which is 1-substituted by carboxyl and 2-substituted by amino and which may at one other position contain an S atom).

25

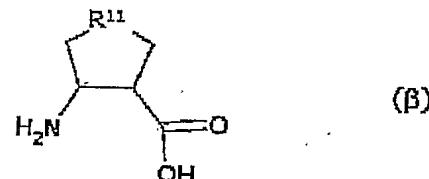
An exemplary class of products comprises those in which aa² is a residue of an imino acid of formula (IV)



30 where R¹¹ is -CH₂-, -CH₂-CH₂-, -CH=CH-, -S-CH₂- or -CH₂-CH₂-CH₂-, which group when the ring is 5 or 6-membered is optionally substituted at one or more -CH₂- groups by from 1 to 3 C₁-C₃ alkyl

groups, for example to form the R¹¹ group -S-C(CH₃)₂- Of these imino acids, azetidine-2-carboxylic acid, especially (s)-azetidine-2-carboxylic acid, and more particularly proline-C(O)OH are illustrative.

5 Also to be mentioned as aa² are β -amino acids of formula β :



wherein R¹¹ is as previously defined.

10 In embodiments, aa² is a residue of an N-substituted imino acid or N-substituted β -amino acid.

It will be appreciated from the above that a very preferred class of products consists of those in which aa¹-aa² is 4-F-Phe-Pro. In another preferred class, aa¹-aa² is di(4-F)-Dpa-Pro. In other products, aa¹-aa² is Cha-Pro or Dcha-Pro. Of course, also included are corresponding product classes in which Pro is replaced by (s)-azetidine-2-carboxylic acid.

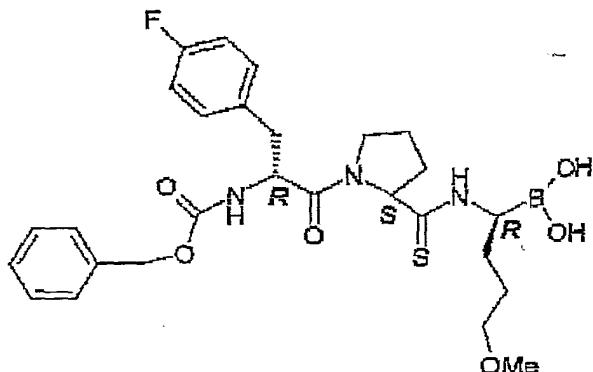
15 R⁹ is as defined previously and may be a moiety R¹ of the formula -(CH₂)_s-Z. Integer s is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, I or, preferably, Br). Particularly illustrative Z groups are -OMe and -OEt, especially -OMe. In certain examples s is 3 for all Z groups and, indeed, for all 20 compounds of the disclosure. Particular R¹ groups are 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 4-bromobutyl, 4-chlorobutyl, 4-methoxybutyl and, especially, 3-bromopropyl, 3-chloropropyl and 3-methoxypropyl. Most preferably, R¹ is 3-methoxypropyl. 2-Ethoxyethyl is another preferred R¹ group.

25 Accordingly, a specific class of compounds consists of those of acids of the formula X-4-F-Phe-Pro-Mpg-B(OH)₂, especially Cbz-4-F-Phe-Pro-Mpg-B(OH)₂; also included are analogues of these compounds in which Mpg is replaced by a residue with another of the R¹ groups and/or 4-F-Phe is replaced by di(4-F)-Dpa or another aa¹ residue. Also included are compounds in which Cbz is replaced by benzylmethylcarbonyl (Ph-Et-CO-).

30

The aa¹ moiety of the product is preferably of R configuration. The aa² moiety is preferably of (S)-configuration. Particularly preferred products have aa¹ of (R)-configuration and aa² of (S)-configuration. The chiral centre -NH-CH(R¹)-B- is preferably of (R)-configuration. It is considered

that commercial formulations will have the chiral centres in (R,S,R) arrangement, as for example in the case of salts of Cbz-(R)-4-F-Phe-(S)-Pro-(R)-boroMpg-OH (TRI 50F):



TRI 50F

The disclosure includes Cbz-(R)-4-F-Phe-(S)-Pro-(R)-boroMpg-OH and Cbz-(R)-di-(4-F)-Dpa-(S)-Pro-(R)-boroMpg-OH (and other compounds of the formula X-(R)-4-F-Phe-(S)-Pro-(R)-boroMpg-OH and X-(R)-di-(4-F)-Dpa-(S)-Pro(R)-boroMpg-OH) which for example are at least 90% pure, e.g. at least 95% pure. These compounds may be in the form of, e.g., base addition salts.

Also disclosed herein are acid addition salts, which may be formed by contacting a disclosed acid with a pharmaceutically acceptable acid, particularly a strong inorganic acid, e.g. HBr, HCl or HSO_2CH_3 . The pharmaceutically acceptable acid will form a salt with an amino group, e.g. an unprotected N-terminal amino group.

In broad terms, the base addition salts described herein may be considered to correspond to reaction products of an organoboronic acid as described above with a strong base, e.g. a basic metal compound; the salts are however not limited to products resulting from such a reaction and may be obtained by alternative routes.

The base addition salts are therefore obtainable by contacting an acid of the disclosure with a strong base. The disclosure thus contemplates products (compositions of matter) having the characteristics of a reaction product of a disclosed acid and a strong base. The base is pharmaceutically acceptable.

As suitable salts may be mentioned salts of metals, e.g. of monovalent or divalent metals, and stronger organic bases, for example:

1. Alkali metal salts;

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2. Divalent, e.g. alkaline earth metal, salts;

3. Group III metals;

5 4. Salts of strongly basic organic nitrogen-containing compounds, including:

4A. Salts of guanidines and their analogues;

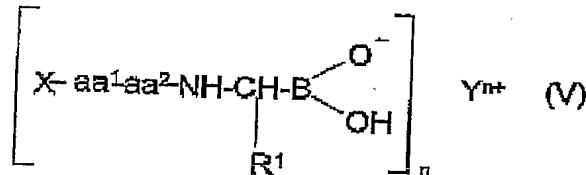
4B. Salts of strongly basic amine, examples of which include (i) aminosugars and (ii) other amines.

10 Of the above salts, particularly illustrative are alkali metals, especially Na and Li. Also illustrative are aminosugars,

15 Specific salts are of the acid boronate though in practice the acid salts may contain a very small proportion of the doubly deprotonated boronate. The term "acid boronate" refers to trigonal $-B(OH)_2$ groups in which one of the B-OH groups is deprotonated as well as to corresponding tetrahedral groups in equilibrium therewith. Acid boronates have a stoichiometry consistent with single deprotonation.

20

The disclosure includes therefore products (compositions of matter) which comprise salts which may be represented by formula (V):



25 where Y^{n+} is a pharmaceutically acceptable cation obtainable from a strong base, and aa^1 , aa^2 , X and R^1 are as defined above. Also included are products in which R^1 is replaced by another R^2 group.

30 One class of salts have a solubility of about 10 mM or more, e.g. of at least about 20mM, when their solubility is determined as described in the examples at a dissolution of 25mg/ml. More particularly yet they have a solubility of least 50mM when their solubility is determined as described in the examples at a dissolution of 50mg/ml.

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The disclosure includes salts of boronic acids (I) having an observed stoichiometry consistent with the salt being of (being representable by) the formula "(boronate)_n cationⁿ⁺". One class of such salts are represented by the formula:



5 where M⁺ represents a monovalent cation, especially an alkali metal cation. It will be understood that the above representation is a notional representation of a product whose observed stoichiometry is unlikely to be literally and exactly 1:1. In any event, a particular salt is Cbz-(R)-4-F-Phe-(S)-Pro-(R)-Mpg-B(OH)₂ monosodium salt. In the above formula, the trigonally-represented boronate represents, as always, boronates which are trigonal, tetrahedral or mixed trigonal/tetrahedral.

10 Particularly exemplary are products which comprise:

(i) species selected from (a) acids of formula (VIII): X-(R)-4-F-Phe-(S)-Pro-(R)-Mpg-B(OH)₂

15 where X is H or an amino-protecting group, especially Cbz, (b) boronate anions thereof, and (c) any equilibrium form of the foregoing (e.g. an anhydride); and

(ii) ions having a valency n in combination with said species, the species and said ions having an observed stoichiometry consistent with a notional species:ion stoichiometry of n:1. In one class of salts, n is 1.

20

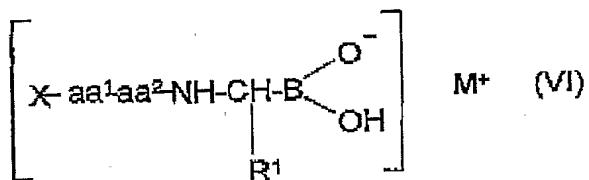
Considering the counter-ions in turn:

1. Monovalent metal, especially alkali metal salts

25 Suitable alkali metals include lithium, sodium and potassium. All of these are remarkably soluble. Lithium and sodium are illustrative because of their high solubility. The lithium and particularly sodium salts are of surprisingly high solubility in relation to potassium amongst others. Sodium is the most used in many instances. Salts containing mixtures of alkali metals are contemplated by the disclosure.

30

The disclosure includes products comprising salts of the formula (VI)



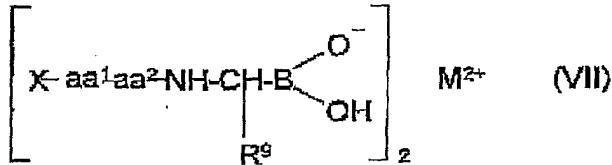
where M^+ is an alkali metal ion; and aa^1 , aa^2 , X and R^1 are as defined above. Also included are salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^+ group) and mixtures of such salts. Included also are products wherein R^1 is replaced by another R^9 group.

5

2. Divalent, e.g. alkaline earth metal (Group II metal) salts

One example of a divalent metal is calcium. Another suitable divalent metal is magnesium. Also contemplated is zinc. The divalent metals are usually used in a boronic acid:metal ratio of substantially 2:1, in order to achieve the preferred monovalent boronate moiety. Salts containing mixtures of divalent metals, e.g. mixtures of alkaline earth metals, are also contemplated.

Further disclosed are products (compositions of matter) which comprise salts which may be represented by the formula (VII):



15

where M^{2+} is a divalent metal cation, e.g. an alkaline earth metal or zinc cation; and aa^1 , aa^2 , X and R^1 are as defined above. Also included are salts in which both hydroxy groups of the boronate group are deprotonated and mixtures of such salts. As previously indicated, the boronate may comprise a tetrahedral species.

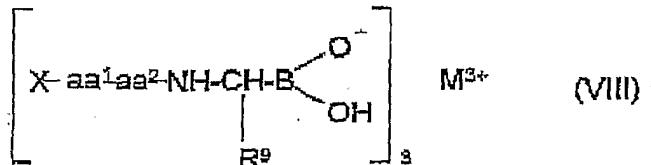
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3. Group III metals

Suitable Group III metals include aluminium and gallium. Salts containing mixtures of Group III metals are also contemplated.

25

The disclosure includes products comprising salts of the formula (VIII):



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where M^{3+} is a Group III metal; and aa^1 , aa^2 , X and R^1 are as defined above. Also included are salts in which both hydroxy groups of the boronate group are in salt form and mixtures of such salts. As previously indicated, the boronate may comprise a tetrahedral species.

5 4. Strongly basic organic nitrogen-containing compounds

The disclosure includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base. Two illustrative classes of organic base are described in sections 4A and 4B below. Particularly preferred are acid 10 salts (in which one of the two boronic -OH groups is deprotonated). Most commonly, the salts contain a single type of organic counter-ion (disregarding trace contaminants) but the disclosure contemplates salts containing mixtures of organic counter-ions; in one sub-class, the different counter-ions all fall within the section 4A family described below or, as the case may be, in the section 2B family below; in another subclass, the salts comprise a mixture of organic counter-ions 15 which are not all from the same family (4A or 4B).

Suitable organic bases include those with a pK_b of 7 or more, e.g. 7.5 or more, for example in the region of 8 or more. Bases which are less lipophilic [e.g. have at least one polar functional group (e.g. 1, 2 or 3 such groups) for example hydroxy] are favoured; thus aminosugars are one favoured 20 class of base.

4A. Guanidines and their analogues

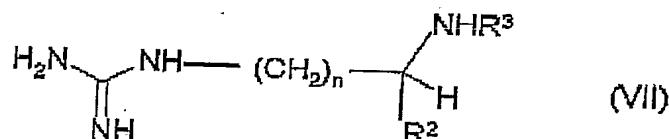
The guanidino compound (guanidine) may in principle be any soluble and pharmaceutically acceptable compound having a guanidino or a substituted guanidino group, or a substituted or 25 unsubstituted guanidine analogue. Suitable substituents include aryl (e.g. phenyl), alkyl or alkyl interrupted by an ether or thioether linkage and, in any event, typically contain from 1 to 6 and especially 1, 2, 3, or 4 carbon atoms, as in the case of methyl or ethyl. The guanidino group may 30 have 1, 2, 3 or 4 substituent groups but more usually has 1 or 2 substituent groups, for instance on a terminal nitrogen. One class of guanidines is monoalkylated; another class is dialkylated. As guanidine analogues may be mentioned thioguanidines and 2-amino pyridines. Compounds having unsubstituted guanidino groups, for example guanidine and arginine, form one particular class.

35 Salts containing mixtures of guanidines are contemplated by the disclosure.

A particular guanidino compound is L-arginine or an L-arginine analogue, for example D-arginine, or the D- or, preferably, L- isomers of homoarginine or agmatine [(4-aminobutyl) guanidine]. Less preferred arginine analogues are NG-nitro-L-arginine methyl ester, for example, and constrained guanidine analogues, particularly 2-amino pyrimidines, for example 2,6-quinazolinediamines such as

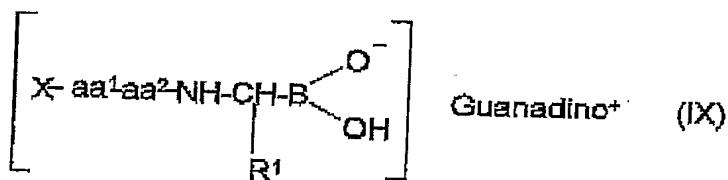
5,6,7,8-tetrahydro-2,6-quinazolinediamine, for example. The guanidino compound may also be a peptide, for example a dipeptide, containing arginine; one such dipeptide is L-tyrosyl-L-arginine.

Some particular guanidino compounds are compounds of formula (VII).



5 where n is from 1 to 6 and for example at least 2, e.g. 3 or more, and in many instances no more than 5. Most particularly, n is 3, 4 or 5. R^2 is H or carboxylate or derivatised carboxylate, for example to form an ester (e.g. a C_1 - C_4 alkyl ester) or amide. R^3 is H, C_1 - C_4 alkyl or a residue of a natural or unnatural amino acid (e.g. tyrosine). The compounds of formula (IV) are usually of L-configuration. The compounds of formula (IV) are arginine ($n=3$; R^2 =carboxyl; R^3 =H) and arginine derivatives or analogues.

The disclosure includes products comprising salts of the formula (IV)



15 where Guanadino⁺ is the protonated form of a pharmaceutically acceptable organic compound comprising a guanidino group or an analogue thereof; and aa¹, aa², X and R¹ are as defined above. Also included are salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical Guanadino⁺ group) and mixtures of such salts. Also included are products wherein R¹ is replaced by another R⁹ group.

20 4B. Strongly basic amines

The disclosure includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base which is an amine. The amine may in principle be any soluble and pharmaceutically acceptable amine.

25

It is envisaged that a desirable class of amine includes those having polar functional groups in addition to a single amine group, as such compounds will be more hydrophilic and thus more soluble than others. In certain salts, the or each additional functional group is hydroxy. Some amines have 1, 2, 3, 4, 5 or 6 additional functional groups, especially hydroxy groups. In one illustrative class of

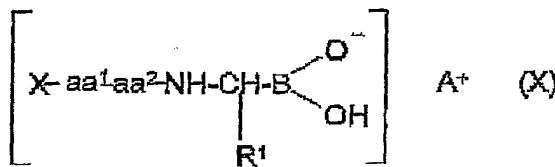
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amines the ratio of (amino plus hydroxy groups):carbon atoms is from 1:2 to 1:1, the latter ratio being particularly preferred. These amines with one or more additional polar functional groups may be a hydrocarbon, especially an alkane, substituted by the amino group and the additional polar group(s). The amino group may be substituted or unsubstituted and, excluding amino substituents, the polar base may contain, for example, up to 10 carbon atoms; usually there are no less than three such carbon atoms, e.g. 4, 5 or 6. Aminosugars are included in this category of polar bases.

5

The disclosure includes products comprising salts of the formula (X)



10 where A⁺ is the protonated form of a pharmaceutically acceptable amine; and aa¹, aa², X and R¹ are as defined above. Also included are salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical A⁺ group) and mixtures of such salts. In one class of such products, A⁺ is the protonated form of an amine described in section 2B(i) below; in another class A⁺ is the protonated form of an amine described in 2B(ii) below. Also included are products in 15 which R¹ is replaced by another R² group.

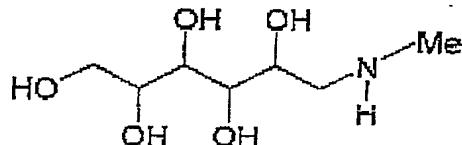
Two illustrative classes of amine base are described in sections 4B(i) and 4B(ii) below. Particularly preferred are acid salts (in which one of the two boronic -OH groups is deprotonated). Most commonly, the salts contain a single type of amine counter-ion (disregarding trace contaminants) 20 but the disclosure contemplates salts containing mixtures of amine counter-ions; in one sub-class, the different counter-ions all fall within the sub-section 4B(i) family described below or, as the case may be, in the sub-section 4B(ii) family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (4B(i) or 4B(ii)).

25 **4B(i) Aminosugars**

The identity of the aminosugar is not critical. Preferred aminosugars include ring-opened sugars, especially glucamines. Cyclic aminosugars are also envisaged as useful. One class of the aminosugars is N-unsubstituted and another, preferred, class is N-substituted by one or two N-substituents (e.g. one). Suitable substituents are hydrocarbyl groups, for example and without limitation containing from 1 to 12 carbon atoms; the substituents may comprise alkyl or aryl moieties or both. Exemplary substituents are C₁, C₂, C₃, C₄, C₅, C₆, C₇ and C₈ alkyl groups, in particular methyl and ethyl, of which methyl is illustrative. Data indicate that aminosugars, especially N-methyl-D-glucamine, are of surprisingly high solubility.

30

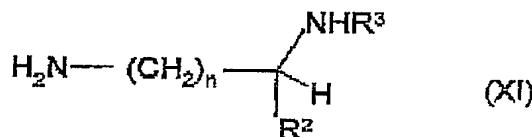
A most preferred aminosugar is N-methyl-D-glucamine:



4B(ii) Other amines

5 Other suitable amines include amino acids (whether naturally occurring or not) whose side chain is substituted by an amino group, especially lysine.

Some amines are compounds of formula (XI):



10 where n , R^2 and R^3 are as defined in relation to formula (IV). The compounds of formula (VI) are usually of L-configuration. The compounds of formula (VI) are lysine ($n=4$; $\text{R}^2=\text{carboxyl}$; $\text{R}^3=\text{H}$) and lysine derivatives or analogues. A most preferred amine is L-lysine.

15 Other suitable amines are nitrogen-containing heterocycles. At least usually, such heterocyclic compounds are alicyclic; one class of the heterocyclic compounds is N-substituted and another, preferred, class is N-unsubstituted. The heterocycles may contain 6 ring-forming atoms, as in the cases of piperidine, piperazine and morpholine. One class of amines includes N-containing heterocycles substituted by polar substituents, especially hydroxy, e.g. 1, 2 or 3 times.

20 The disclosure therefore includes amines other than aminosugars which have one or more (e.g. 1, 2, 3, 4, 5 or 6) polar substituents, especially hydroxy, in addition to one amine group. Such compounds may have a ratio of (amino plus hydroxy groups):carbon atoms of 1:2 to 1:1, the latter ratio being particularly preferred.

25 The disclosure includes mixed salts, i.e. salts containing a mixture of boropeptide moieties and/or counterions but single salts are preferred.

The salts in solid form may contain a solvent, e.g. water. There are included a class of products in which the salts are essentially anhydrous. Also included is a class in which the salts are hydrates.

30

Synthetic Methods

1. Peptide/Peptidomimetic Synthesis

5 The synthesis of boropeptides, including, for example, Cbz-D-Phe-Pro-BoroMpg-OPinacol is familiar to those skilled in the art and described in the prior art mentioned above, including Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338). It is described also by Elgendi et al *Adv. Exp. Med. Biol. (USA)* 340:173-178, 1993; Claeson, G. et al *Biochem.J.* 290:309-312, 1993; Deadman et al *J. Enzyme Inhibition* 9:29-41, 1995, and by Deadman et al *J. Med. Chem.* 38:1511-1522, 1995.

10

By way of example, a P2 amino acid, e.g. proline, is coupled with a compound which will form the P3 residue, e.g. Cbz-4-fluorophenylalanine (commercially available), under peptide coupling conditions, to form a dipeptide.

15

The end product dipeptide is reacted with an aminoboronate corresponding to the desired P1 residue, e.g. boroMpg, to form the final product

20

Stereoselective synthesis with S or R configuration at the chiral B-terminal carbon may be conducted using established methodology (Elgendi et al *Tetrahedron Lett.* 33:4209-4212, 1992; WO 92/07869 and family members including US 5648338) using (+) or (-)- pinanediol as the chiral director (Matteson et al *J. Am. Chem. Soc.* 108:810-819, 1986; Matteson et al *Organometallics.* 3:1284-1288, 1984). Another approach is to resolve the requisite aminoboronate intermediate (e.g. Mpg-BOPinacol) to selectively obtain the desired (R)-isomer and couple it to the dipeptide moiety (e.g. Cbz-(R)-4-F-Phe-(S)-Pro) which will form the remainder of the molecule.

25

For additional information on peptide synthesis, the skilled reader is referred to "The Peptides", Vol. 1-3, Edited by Erhard Gross, Johannes Meienhofer, 1979 (Vol.1), 1980(Vol.2), 1981(Vol.3), Academic Press.

30

The boropeptides may be synthesised initially in the form of boronic acid esters, particularly esters with diols. Such diol esters may be converted to the peptide boronic acid as described next.

2. Ester to Acid Conversion

35

A peptide boronate ester such as Cbz-(R)-4-F-Phe-Pro-boroMpg-OPinacol may be hydrolysed to form the corresponding acid.

A novel technique for converting a diol ester of a peptide boronic acid of formula (I) into the acid comprises dissolving the diol ester in an ether and particularly a dialkyl ether, reacting the thus-

dissolved diol with a diolamine, for example a dialkanolamine, to form a product precipitate, recovering the precipitate, dissolving it in a polar organic solvent and reacting the thus-dissolved product with an aqueous medium, e.g. an aqueous acid, to form the peptide boronic acid. The boronic acid may be recovered from the organic layer of the mixture resulting from the reaction, for example by removing the solvent, e.g. by evaporation under vacuum or distillation. The reaction between the diol ester and the diolamine may be carried out under reflux, for example.

The identity of the diol is not critical. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting carbon atoms in a chain or ring. One class of diols comprises hydrocarbons substituted by exactly two hydroxy groups. One such diol is pinacol and another is pinanediol; there may also be mentioned neopentylglycol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

The alkyl groups of the dialkyl ether preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. An exemplary ether is diethyl ether.

The alkyl groups of the dialkanolamine preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. An exemplary dialkanolamine is diethanolamine. The diethanolamine/boronic acid reaction product hydrolyses in water at room temperature and the rate of hydrolysis may be accelerated by adding acid or base.

The polar organic solvent is preferably CHCl_3 . Other examples are polyhalogenated alkanes generally and ethyl acetate. In principle, any polar organic solvent is acceptable other than alcohols.

The aqueous acid is suitably a strong inorganic acid at a pH in the region of 1 such as hydrochloric acid, for example.

After reaction with the acid, the reaction mixture is suitably washed with, for example, NH_4Cl or another mild base.

An example of a specific procedure is as follows

1. The pinacol or pinanediol ester of the selected peptide boronic acid is dissolved in diethyl ether.
2. Diethanolamine is added and the mixture is refluxed at 40 °C.
3. The precipitated product is removed (filtered), washed (usually several times) with diethyl ether or another polar organic solvent other than an alcohol, and dried (e.g. by evaporation under vacuum).

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4. The dry product is dissolved in a polar organic solvent other than an alcohol, e.g. CHCl_3 . Aqueous acid or base is added, e.g. hydrochloric acid (pH 1), and the mixture is stirred for e.g. approximately 1h at room temperature.
5. The organic layer is removed and washed with NH_4Cl solution.
- 5 6. The organic solvent is distilled off and the residual solid product is dried.

The above process results in the formation of what may conveniently be referred to as a "diolamine adduct" of the peptide boronic acids of formula (I), especially such adducts with diethanolamine, and such adducts are themselves included in the disclosure. The molecular structure of such adducts is 10 not known: they might comprise a compound in which the two oxygens and the nitrogen of the diolamine are all coordinated to the boron; they might comprise ions. The adducts are however considered to be esters. A particular novel product included in the disclosure is that obtainable by reacting a pinacol or pinanediol ester of a compound of Formula VIII, particularly Cbz-(R)-4-F-Phe-(S)-Pro-(R)-boroMpg.

15

It will be appreciated that the foregoing technique comprises an example of a method for recovering an organoboronic acid product, the method comprising providing in a solvent a dissolved mixture comprising the organoboronic acid in a soluble form and a compound having two hydroxy groups and an amino group (i.e. a diolamine), causing or allowing the organoboronic acid and the diolamine to react to form a precipitate, and recovering the precipitate. The soluble form of the organoboronic acid may be a diol ester, as discussed above. The solvent may be an ether, as discussed above. The organoboronic acid may be one of the organoboronic acids referred to in this specification, for example it may be of Formula (I) or (III). The method described in this paragraph is novel and forms an aspect of the disclosure. A recovery method is filtration.

25

The reaction between the diolamine and the soluble form of the organoboronic acid is suitable carried out at an elevated temperature, for example under reflux.

30

Another aspect of the disclosure is a method for recovering an organoboron species, comprising providing, in a form soluble in an ether, an organoboronic acid, for example a drug such as, e.g., a compound of formula (III); forming a solution of the soluble form in the ether; combining the solution with a dialkanolamine and allowing or causing the dialkanolamine to react with the soluble form of the organoboronic acid to form an insoluble precipitate; and 35 recovering the precipitate.

The term "soluble" in the preceding paragraph refers to species which are substantially more soluble in the reaction medium than is the precipitated product. In variants of the method, the ether is replaced by toluene or another aromatic solvent.

The diethanolamine precipitation technique described above is an example of another novel method, which is a method for recovering from ether solution a pinacol or pinanediol ester of a peptide boronic acid, comprising dissolving diethanolamine in the solution, allowing or causing a precipitate to form and recovering the precipitate. The disclosure encompasses variants of this methods in which another diol than pinacol or pinanediol is used.

5 The precipitated material, i.e. the "adduct", may be converted into the free organoboronic acid, for example by contacting it with an acid. The acid may be an aqueous acid, for example an aqueous 10 inorganic acid, e.g. as described above. The precipitate may be dissolved, for example in an organic solvent, prior to being contacted with the acid.

15 The disclosure therefore provides a method for making an organoboronic acid, comprising converting its diolamine reaction product to the acid.

15 The acid resulting from the methods described in the previous two paragraphs may be converted to a salt of the acid with a multivalent metal, which salt may in turn be formulated into a pharmaceutical composition in parenteral dosage form.

20 3. Salt Synthesis

Acid addition salts may be prepared by contacting the boronic acid compound with a pharmaceutically acceptable acid, notably a strong acid.

25 In general, the base addition salts may be prepared by contacting the relevant peptide boronic acid with a strong base appropriate to form the desired salt. In the case of metal salts, the metal hydroxides are suitable bases (alternatively, metal carbonates might be used, for example), whilst sometimes it is more convenient to contact the acid with a relevant metal alkoxide (e.g. methoxide), for which purpose the corresponding alkanol is a suitable solvent. Salts with organic bases may be 30 prepared by contacting the peptide boronic acid with the organic base itself. Illustrative salts are acid salts (one -BOH proton replaced) and, to make acid salts with a monovalent cation, the acid and the base are suitably reacted in substantially equimolar quantities. Generally stated, therefore, the usual acid:base molar ratio is substantially n:1, where n is the valency of the cation of the base.

35 In one procedure, a solution of the peptide boronic acid in a water-miscible organic solvent, for example acetonitrile or an alcohol (e.g. ethanol, methanol, a propanol, for example iso-propanol, or another alkanol), is combined with an aqueous solution of the base. The acid and the base are allowed to react and the salt is recovered. The reaction is typically carried out at ambient temperature (e.g. at a temperature of from 15 to 30°C, e.g. 15 to 25°C), but an elevated

temperature may be used, for example up to the boiling point of the reaction mixture but more usually lower, e.g. a temperature of up to 40°C or 50°C. The reaction mixture may be allowed to stand or be agitated (usually stirred).

5 The time during which the acid and the base are allowed to react is not critical but it has been found desirable to maintain the reaction mixture for at least one hour. A period of from one to two hours is usually suitable but longer reaction times may be employed.

10 The salt may be recovered from the reaction mixture by any suitable method, for example evaporation or precipitation. Precipitation may be carried out by adding an excess of a miscible solvent in which the salt has limited solubility. In one preferred technique, the salt is recovered by evacuating the reaction mixture to dryness. The salt is preferably thereafter purified, for example by redissolving the salt before filtering the resulting solution and drying it, for example by evacuating it to dryness. The redissolution may be performed using water, e.g. distilled water. The salt may then 15 be further purified, for example in order to remove residual water by further redissolution in a suitable solvent, which is advantageously ethyl acetate or THF followed by evaporating to dryness. The purification procedure may be carried out at ambient temperature (say, 15 to 30°C, e.g. 15 to 25°C), or at a modestly elevated temperature, such as e.g. a temperature not exceeding 40°C or 50°C; for example the salt may be dissolved in water and/or solvent by agitating with or without 20 warming to, for example, 37°C.

Also included is a method for drying the salts of the disclosure and other peptide boronic acid salts, comprising dissolving them in an organic solvent, e.g. ethyl acetate or THF, and then evaporating to dryness, e.g. by evacuation.

25 Generally, preferred solvents for use in purifying the salts are ethyl acetate or THF, or perhaps another organic solvent.

A general procedure for synthesising salts of Cbz-4-F-Phe-Pro-BoroMpg-OH is as follows:

30 Cbz-4-F-Phe-Pro-BoroMpg-OH (38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added the requisite base in solution in distilled water (190ml); the base is added as a 0.2M solution for a monovalent cation. The resultant clear solution is allowed to react for example by being left to stand or being agitated, for a usual period, in either case, of from 35 one to two hours. The reaction is typically carried out at ambient temperature (e.g. 15-30°C, e.g. 15 to 25°C) but alternatively the temperature may be elevated (e.g. up to 30°C, 40°C or 50°C). The reaction mixture is then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The product is redissolved in the minimum amount of distilled water necessary (200ml to 4L), typically with warming (e.g. to 30-40°C), usually for up to 2 hours. The solution is filtered, suitably

through filter paper, and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a solid. If the product is present as an oil or tacky solid then it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a solid.

5

In variations of the foregoing general procedure, the acetonitrile is replaced by another water-miscible organic solvent, notably an alcohol, as discussed above, especially ethanol, methanol, isopropanol or another propanol.

10 Where a boronic acid salt is less soluble in a selected reaction medium for salt formation such that its direct preparation from the corresponding acid and base is inconvenient, the less soluble salt may be prepared from a salt more soluble in the reaction medium.

15 There is provided also the use of a boronic acid to make a salt of the disclosure. Included also is a method of preparing a product of the disclosure, comprising contacting a boronic acid, e.g. of formula (I), (II) or (III), with a base capable of making such a salt.

20 The peptide boronic acid of formula (I) used to prepare the pharmaceutical preparations is typically of GLP or GMP quality, or in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice); such acids are included in the disclosure.

25 Similarly the acids are usually sterile and/or acceptable for pharmaceutical use, and one aspect of the disclosure reside in a composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I). Such a composition of matter may be in particulate form or in the form of a liquid solution or dispersion.

The acid may be in isolated form and such isolated acids are included in the disclosure.

4. Separation of Stereoisomers

30

The stereoisomers of a peptide boronic ester or a synthetic intermediate aminoboronate may be resolved in, for example, any known way. In particular, stereoisomers of boronic esters may be resolved by HPLC.

35 5. Specific Synthesis

This method addresses the problems of controlling C-B bond cleavage in organoboronic compounds as well as providing chirally purified on a commercial scale. In this regard, it has been found that C-

B bonds seem to be cleaved by a non-oxidative mechanism which occurs in the presence of many solvents, including water and e.g. aqueous acids and bases, amongst others.

Chirally-selective precipitation may be used to recover organoboronic acids in high purity.

5

Thus C-B bond cleavage may be controlled by:

- Selection of acetonitrile as a solvent, where a solvent is required in processing and acetonitrile has the necessary solvation power; in particular acetonitrile is selected in process where a polar solvent is desirable or necessary.
- 10 • Avoiding excessive contact with water.

In terms of tripeptide boronate salt production, therefore, the disclosure includes processes comprising one, two or three of the following features:

(i) resolution of the (R,S,S) and (R,S,R) epimers of the borotripeptide by chirally selective precipitation using diethanolamine and conveniently, but not necessarily, using as starting material TRI 50F in the form of an ester, for example the pinacol ester;

15 (ii) control of the duration and/or conditions of hydrolysis of the borotripeptide diethanolamine ester, for example as obtained by such precipitation, to control C-B bond breakage;

20 (iii) use of acetonitrile as solvent for the borotripeptide, for example as obtained by such hydrolysis, for the purposes of reacting the borotripeptide with a base to form the salt. Another favourable solvent can be tetrahydrofuran.

25

As an optional, or even stand-alone, fourth feature, the borotripeptide base addition salts may be dried by azeodrying using acetonitrile.

It is considered that C-B bond cleavage may occur by a nucleophilic mechanism, and the disclosure 30 therefore includes methods in which opportunities for nucleophilic attack are minimised.

The above four features, or any one, two or three of them, may be applied to the manufacture and processing of other boronic compounds, particularly acids of formula (I) and their derivatives (e.g. esters and salts).

35

The disclosure provides in one aspect, therefore, the use of diethanolamine to resolve by selective precipitation the diastereomers of the disclosed boronic acids, exemplified below by reference to boronic acids of formula (I). The starting material may be an acid (I) or a derivative thereof capable of forming a diethanolamine ester of the boronic acid. The precipitation selects acids having a chiral

centre C* of (R) configuration as precipitate. The precipitate may be recovered and converted to the corresponding boronic acid or a salt thereof. The salt may be made into a pharmaceutical formulation.

5 For optimised chiral purity and yield, the diethanolamine may be used in an amount of about 1.25 ± 0.1 equivalents based on initial equivalents of boronic acid having a chiral centre C* of (R) configuration.

10 The initial boronic acid or acid derivative may for example comprise from 50% to 60% molecules having chiral centre C* of (R)-configuration and from 40% to 50% molecules having chiral centre C* of (S)-configuration.

15 The method opens the way to commercialisation of the boronic acids (I) and their derivatives, particularly salts, as pharmaceuticals. Commercial scale products and activities using the boronic acids (I) and their derivatives are therefore provided.

In one embodiment, there is provided a process for separating diastereomers of a boronic acid of formula (I), comprising:

20 combining in diethylether solution (A) a boronic species selected from the boronic acid (I) and its esters, the boronic species including molecules having a chiral centre C* of (R) configuration and molecules having a chiral centre C* of (S) configuration, and (B) diethanolamine, the diethanolamine being in an amount of about 1.25 ± 0.1 equivalents based on the boronic species in which the chiral centre C* is of (R) configuration, and mixing to form a mixture;

25 causing or allowing the boronic species and the diethanolamine to react until a precipitate forms; and

recovering the precipitate.

When the starting material is an ester, it may be an ester of the boronic acid with an alcohol selected from the group consisting of alcohols whose sole potential electron donor heteroatoms are 30 oxygens which, in the boronic ester, correspond to the oxygens of the ester functional group.

In some methods, the diethanolamine is in an amount of from 1.2 to 1.3 equivalents based on the boronic species in which chiral centre C* is of (R) configuration.

35 There are included processes in which the boronate species is an ester of the boronic acid and a diol, in particular a diol which is not sterically hindered. As exemplary diols may be mentioned pinacol, neopentylglycol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, or 5,6-decanediol. A particular diol is pinacol.

The boronic species and the diethanolamine may be caused to react by heating the mixture to an elevated temperature, for example the mixture may be refluxed, e.g. for at least 10 hours.

5 The precipitate may be recovered by filtration. The recovered precipitate may be washed with diethylether. The recovered precipitate, after washing if such takes places, may be dissolved in a solvent selected from CH_2Cl_2 and CHCl_3 and reprecipitated by combining the resulting solution with diethylether. A particular solvent is CH_2Cl_2 .

10 The recovered precipitate may be converted to the acid of formula (I), suitably by hydrolysis, for example by dissalving the precipitate in an organic solvent selected from e.g. halohydrocarbons and combinations thereof, agitating the resulting solution with an aqueous liquid, e.g. an aqueous acid having a pH of below 3, whereby the dissolved precipitate is converted to the formula (I) acid, and recovering the formula (I) acid by evaporation. The organic solvent may be CH_2Cl_2 or CHCl_3 . A particular solvent is CH_2Cl_2 . In some processes, organic solvent is further evaporated from the 15 recovered formula (I) acid.

20 The disclosure includes methods in which an ester of a boronic acid (I), particularly a diethanolamine ester, is hydrolysed in a manner which controls C-B bond cleavage. In particular, this involves limiting the period of hydrolysis at the selected temperature. In the case of diethanolamine ester hydrolysis, the hydrolysis is suitably carried out at room temperature, or less, for a period not exceeding about 30 minutes, e.g. not exceeding about 20 minutes, and optimally of about 20 minutes.

25 Thus the recovered precipitate referred to in the last paragraph but one may be hydrolysed using an aqueous acid, particularly 2% hydrochloric acid or another mineral acid of similar pH, for no more than about 30 minutes at about room temperature, or less. Suitably, the precipitate is dissolved in a non-nucleophilic organic solvent (e.g. a halohydrocarbon or halohydrocarbon mixture for example CH_2Cl_2) and the resulting solution is contacted with the aqueous acid for a period as previously described. The precipitate is thereby hydrolysed to form the free acid of formula (I), which remains 30 in the organic solvent. The organic solvent may be separated from the aqueous medium and then evaporated to obtain solid acid of formula I.

35 There are included processes in which a formula (I) acid, for example obtained as described in the preceding paragraph, is dried. In a class of processes, the formula (I) acid is dried when it is in the organic solvent by contacting the solvent with a hygroscopic solid.

Included are processes in which the formula (I) acid, when in the organic solvent, is washed with an aqueous ammonium salt.

Chirally purified boronic acid may be converted to a pharmaceutically acceptable base addition salt thereof, in particular by dissolving the acid in acetonitrile, combining the resultant solution with an aqueous solution or suspension of a pharmaceutically acceptable base, and causing or allowing the base and the acid to react, then evaporating to dryness to obtain an evaporation residue. The step of causing or allowing the acid and the base to react may comprise agitating the combination of the acetonitrile solution of the acid and the aqueous solution or suspension of the base at a temperature of not more than 35°C and often of not more than 30°C, e.g. not more than 25°C; an optimal temperature is room temperature, in which case a reaction time of about 2 hours might be appropriate. The process may further comprise:

- (i) redissolving the evaporation residue in acetonitrile and evaporating the resulting solution to dryness; and
- (ii) repeating step (i) as often as necessary to obtain a dry evaporation residue.

In some processes the dry evaporation residue is dissolved in acetonitrile or tetrahydrofuran to form a solution, and the solution is combined with (e.g. slowly added to, at a rate sufficiently slow to avoid lump formation) a 3:1 to 1:3 v/v mixture of diethylether and an aliphatic or cycloaliphatic solvent to form a precipitate, said solution being added to the diethylether/(cyclo)aliphatic solvent mixture in a ratio (solution:mixture) of from 1:5 to 1:15 v/v. The precipitate is recovered and some or substantially all remaining solvent is removed from the recovered precipitate whilst maintaining the temperature at no more than 35°C, e.g. is removed under reduced pressure. Included are processes in which the temperature at the start of the drying process is about 10°C and is increased during the process to 35°C. The aliphatic or cycloaliphatic solvent may have 6, 7 or 8 carbon atoms; the solvent may be an alkane, for example an n-alkane, e.g. n-heptane. Some reactions may be carried out at ambient temperature, which may e.g. be 15-30°C, e.g. 20-30°C; sometimes ambient temperature may be room temperature.

The salts produced by the invention may contain a trace amount of the aliphatic or cycloaliphatic solvent, e.g. an amount of less than 0.1%, particularly less than 0.01%, for example an amount of about 0.005%.

In the process for making the salt, the base may comprise a cation of valency n and be used in a stoichiometry (boronic acid:base) of about n:1. In particular processes, the base is an alkali metal or alkaline earth metal base, for example an alkali metal hydroxide or an alkaline earth metal hydroxide. As one base may be mentioned sodium hydroxide. As another base may be mentioned calcium hydroxide. The disclosure includes processes in which the base is sodium hydroxide and the dry evaporation residue is dissolved in acetonitrile. The disclosure includes processes in which the base is calcium hydroxide and the dry evaporation residue is dissolved in tetrahydrofuran.

The disclosure is not limited as to the method by which the boronic acids of Formula (I) are obtained (for example as an ester thereof). However, in one class of subject matter, the Formula (I) acid has an R¹ group of the formula -(CH₂)_s-O-R³ in which R³ is methyl or ethyl and s is independently 2, 3 or 4, and the Formula (I) acid is prepared via an intermediate of Formula (XXV):



which intermediate is made by reaction between a borate ester and a suitable 1-metallocalkoxyalkane.

A novel aspect of the disclosure comprises the Formula (XXV) intermediates.

10

The Formula (XXV) intermediates may be made by reacting a 1-metallocalkoxyalkane, where the alkoxyalkane is of the formula -(CH₂)_s-O-R³, with a borate ester to form a compound of Formula (XXV).

15

It will be appreciated that the above method provides a general procedure for making alkoxyalkylboronic acids, which may be presented by the formula R^Z-O-R^Y-B(OH)₂. Such alkoxyalkylboronic acids may be converted to aminoboronates, and the aminoboronates may be derivatised at their amino group to form an amide bond linked to another moiety. In other words, the aminoboronates may be converted to boropeptides. The method will now be described further 20 with non-limiting reference to compounds of Formula (XXV).

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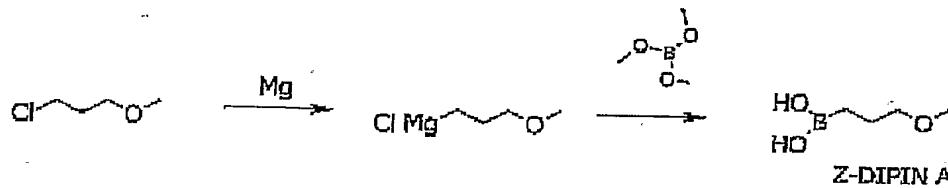
The starting materials for the reaction may be a metallocalkoxyalkane, e.g. a Grignard reagent, obtainable from 1-haloalkoxyalkane of the formula Hal-(CH₂)_s-O-R³ (where Hal is a halogen) and a borate ester. The metal is in particular magnesium. Another metal is lithium, in which case the metalloc reagent may be prepared by reacting the 1-haloalkoxyalkane with butyl lithium. Where the method includes preparation of the metalloc reagent from the haloalkoxyalkane, the haloalkoxyalkane may be a chloroalkoxyalkane; the corresponding bromo compounds may also be used. To make a Grignard reagent, magnesium may be reacted with the haloalkoxyalkane.

30

Suitable borate esters are esters of mono- and di-functional alcohols (e.g. of EtOH, MeOH, BuOH, pinacol, glycol, pinanediol etc). For example, the ester may be of the formula B(OR^a)(OR^b)(OR^c) where R^a, R^b and R^c are C₁-C₄ alkyl and may be the same as each other.

35

An exemplary procedure for making a Formula (XXV) intermediate, illustrated with reference to methoxypropane as the alkoxyalkane species, is:



The reactions are suitably carried out in an organic solvent, e.g. THF.

The above-described procedure for making alkoxyalkylboronic acids avoids generation of Impurity IV

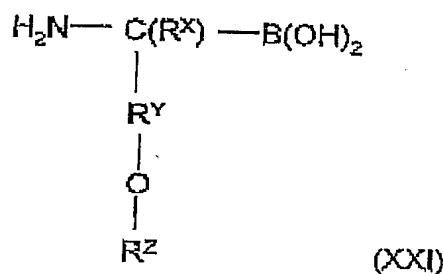
5 (see above), or its analogues in those cases where the end product is not TRI 50F or a derivative (salt, ester etc) thereof. The procedure therefore provides a unique route to making TRI 50F, its esters and salts, uncontaminated by Impurity IV, and for making other aminoboronic acids which are substituted α - to the boron by an alkoxyalkyl group and are uncontaminated by impurities analogous to Impurity IV.

10

An alkoxyalkylboronic acid, i.e. a compound which may be represented by the formula $R^Z-O-R^Y-B(OH)_2$, may be converted to an aminoboronic compound, for example a boropeptide, by any suitable procedure, e.g. one known in the art. A reaction scheme for making alkoxyalkylboronic acids into aminoboronates, and for converting aminoboronates into peptide boronates is illustrated

15 with reference to synthesis of TRI 50F at the start of the Examples of this specification. The reaction scheme may be modified as desired, e.g.: diethanolamine precipitation and subsequent steps may be omitted, and/or reagent substitutions may be made. For example, pinacol may be replaced by another diol. LDA is a non-nucleophilic strong base and may be replaced by another such base. Other examples include, but are not limited to, lithium diisopropylamide, lithium 2,2,6,6-
20 tetramethylpiperidine, 1-lithium 4-methylpiperazide, 1,4-dilithium piperazide, lithium bis(trimethylsilyl) amide, sodium bis(trimethylsilyl)amide, potassium bis(trimethylsilyl)amide, isopropyl magnesium chloride, phenyl magnesium chloride, lithium diethylamide, and potassium tert-butoxide. The reactions may be carried out in any suitable solvent: where n-heptane is used in the Examples, it may be replaced by another inert non-polar solvent, e.g. another aliphatic or
25 cycloaliphatic solvent, for example an alkane, e.g. an n-alkane.

The product is an aminoboronate of Formula (XXI)



30 wherein

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42

R^X is H or a substituent which does not prevent synthesis;

R^Y is alkylene; and

R^Z is alkyl,

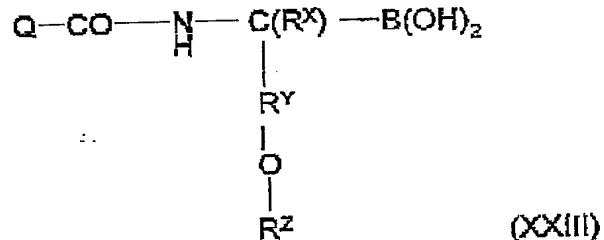
the process comprising reacting a 1-metallocalkoxyalkane with a borate ester to form a boronic acid of the formula $R^Z-O-R^Y-B(OH)_2$, esterifying the acid, contacting the esterified acid with CH_2Cl_2 and $ZnCl_2$ in the presence of a strong base, contacting the resultant product with LiHMDS and in turn contacting the resultant product with hydrogen chloride.

The product is free of contaminant of Formula (XXII):

10 $H_2N-C(R^X)(R^Y)-B(OH)_2$ (XXII).

The aminoboronic (XXI) may be reacted with an amino acid or peptide (which in either case may be suitably protected) to form a peptide boronate. In general terms, therefore, the disclosure includes peptidoboronic acids of Formula (XXIII):

15



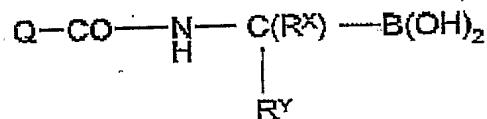
$Q-CO$ comprises at least an amino acid residue;

R^X is H or a substituent which does not prevent synthesis;

R^Y is alkylene;

20 R^Z is alkyl,

which organoboronic acid is free of an impurity of Formula (XXIV):



(XXIV)

25 The disclosure further includes derivatives of Formula (XXIII) acids (e.g. acid or base addition salts, esters) which are free of Formula (XXIV) impurity and derivatives thereof.

The exact identity of R^Y and R^Z is dependent on the identity of the end product, and not part of the process or its benefits.

It will be appreciated from the foregoing that the above described methods may be used in the manufacture of organoboronic acids salts as described. It is not necessary for sequential steps to be carried out as one operation or at the same site; they may be performed in this way or different processes (different parts of the overall synthesis) may be distributed in time and/or space. Particular end product salts are monosodium, monolithium, hemicalcium and hemimagnesium salts, for example of TRI 50S.

10

Generally, the reactions may suitably be carried out with a non-nucleophilic solvent. Where a nucleophilic solvent is present, minimum contact is preferred, for example in the case of hydrolysis of diethanolamine esters.

15

6. Products of Specific Synthesis 5

The products of the invention include *inter alia* boronic acids, diethanolamine esters and salts obtainable by (having the characteristics of a product obtained by) the disclosed methods. Also included are products obtained directly or indirectly by the disclosed methods.

20

Particular products of the invention are base addition salts of a boronic acid of formula (I) having the chiral purity of such salt when prepared by a method described herein. Other products are base addition salts of a boronic acid of formula (I) having the purity of such salt when prepared by a method described herein.

25

Product identities will be apparent from the preceding description and the following examples. In addition, products of the disclosure are described in the claims. Of particular note are the data in Example 9, indicating that the processes of the invention can remarkably achieve end product salts free of impurities detectable by HPLC. In other instances, the salts are substantially free of impurities, e.g. at least 98% pure, more usually at least 99% pure, e.g. at least 99.5% pure, in terms of reverse phase (RP) HPLC percentage peak area. Salts may at least 99.3%, 99.4%, 99.5% 99.6%, 99.7%, 99.8% or 99.9% pure, in terms of reverse phase (RP) HPLC percentage peak area. Suitable RP HPLC procedures comply with reference 1 and/or reference 2 and/or reference 3 of Example 43. Included also are products at least substantially free of Impurity I and analogues, products free of Impurity IV and analogues, and products containing small traces of non-polar solvent, e.g. n-heptane. The trace amount of non-polar solvent may be less than 0.2%, 0.1%, 0.05%, 0.01% or 0.005% as determined by GC-headspace chromatography.

Included also are salts containing less than 410 ppm acetonitrile.

Some salts contain impurities of less than 10,000 ppm, 5000 ppm, 1000 ppm, or 500 ppm.

Use of the Products of the Disclosure

5

The compounds of the disclosure are thrombin inhibitors. They are therefore useful for inhibiting thrombin. There are therefore provided compounds which have potential for controlling haemostasis and especially for inhibiting coagulation, for example in the treatment or prevention of secondary events after myocardial infarction. The medical use of the compounds may be prophylactic 10 (including to treat thrombosis as well as to prevent occurrence of thrombosis) as well as therapeutic (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

The compounds may be employed when an anti-thrombogenic agent is needed. Further, it has been 15 found that the compounds, including those of boronic acids of Formula (III), are beneficial in that the class is useful for treating arterial thrombosis by therapy or prophylaxis. The disclosed compounds are thus indicated in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man. The term "thrombosis" includes *inter alia* atrophic thrombosis, arterial thrombosis, cardiac thrombosis, coronary thrombosis, creeping thrombosis, infective thrombosis, mesenteric thrombosis, placental thrombosis, propagating thrombosis, 20 traumatic thrombosis and venous thrombosis.

It is known that hypercoagulability may lead to thromboembolic diseases.

Examples of venous thromboembolism which may be treated or prevented with compounds of the 25 disclosure include obstruction of a vein, obstruction of a lung artery (pulmonary embolism), deep vein thrombosis, thrombosis associated with cancer and cancer chemotherapy, thrombosis inherited with thrombophilic diseases such as Protein C deficiency, Protein S deficiency, antithrombin III deficiency, and Factor V Leiden, and thrombosis resulting from acquired thrombophilic disorders such as systemic lupus erythematosus (inflammatory connective tissue disease). Also with regard to 30 venous thromboembolism, compounds of the disclosure are useful for maintaining patency of indwelling catheters.

Examples of cardiogenic thromboembolism which may be treated or prevented with compounds of the disclosure include thromboembolic stroke (detached thrombus causing neurological affliction 35 related to impaired cerebral blood supply), cardiogenic thromboembolism associated with atrial fibrillation (rapid, irregular twitching of upper heart chamber muscular fibrils), cardiogenic thromboembolism associated with prosthetic heart valves such as mechanical heart valves, and cardiogenic thromboembolism associated with heart disease.

Examples of conditions involving arterial thrombosis include unstable angina (severe constrictive pain in chest of coronary origin), myocardial infarction (heart muscle cell death resulting from insufficient blood supply), ischemic heart disease (local ischemia due to obstruction (such as by arterial narrowing) of blood supply), reocclusion during or after percutaneous transluminal coronary angioplasty, restenosis after percutaneous transluminal coronary angioplasty, occlusion of coronary artery bypass grafts, and occlusive cerebrovascular disease. Also with regard to arterio-venous (mixed) thrombosis, anti-thrombotic compounds of the disclosure are useful for maintaining patency in arteriovenous shunts.

5 10 Other conditions associated with hypercoagulability and thromboembolic diseases which may be mentioned inherited or acquired deficiencies in heparin cofactor II, circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemia, heparin induced thrombocytopenia and defects in fibrinolysis.

15 20 Particular uses which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism. Preferred indications envisaged for the products of the disclosure (notably the compounds of TRI 50F) include:

- Prevention of venous thromboembolic events (e.g. deep vein thrombosis and/or pulmonary embolism). Examples include patients undergoing orthopaedic surgery such as total hip replacement, total knee replacement, major hip or knee surgery; patients undergoing general surgery at high risk for thrombosis, such as abdominal or pelvic surgery for cancer; and in patients bedridden for more than 3 days and with acute cardiac failure, acute respiratory failure, infection.
- Prevention of thrombosis in the haemodialysis circuit in patients, in patients with end stage renal disease.
- Prevention of cardiovascular events (death, myocardial infarction, etc) in patients with end stage renal disease, whether or not requiring haemodialysis sessions.
- Prevention of venous thrombo-embolic events in patients receiving chemotherapy through an indwelling catheter.
- Prevention of thromboembolic events in patients undergoing lower limb arterial reconstructive procedures (bypass, endarterectomy, transluminal angioplasty, etc).
- Treatment of venous thromboembolic events.
- Prevention of cardiovascular events in acute coronary syndromes (e.g. unstable angina, non Q wave myocardial ischaemia/infarction), in combination with another cardiovascular agent, for example aspirin (acetylsalicylic acid; aspirin is a registered trade mark in Germany), thrombolytics (see below for examples), antiplatelet agents (see below for examples).
- Treatment of patients with acute myocardial infarction in combination with acetylsalicylic acid, thrombolytics (see below for examples).
- Prevention of thrombosis in extracorporeal liver detoxification.

- Flight DVT.
- Preventing thrombosis during coronary artery bypass graft (CABG).
- Prevention of thrombosis in extracorporeal blood circuits generally.

5 The thrombin inhibitors of the disclosure are thus indicated both in the therapeutic and/or prophylactic treatment of all the aforesaid disorders.

In one method, the products of the disclosure are used for the treatment of patients by haemodialysis, by providing the product in the dialysis solution, as described in relation to other thrombin inhibitors in WO 00/41715. The disclosure therefore includes dialysing solutions and dialysing concentrates which comprise a product of the disclosure, as well as a method of treatment by dialysis of a patient in need of such treatment, which method comprises the use of a dialysing solution including a low molecular weight thrombin inhibitor. Also included is the use of an anti-thrombotic product of the disclosure for the manufacture of a medicament for the treatment by dialysis of a patient, in which the anti-thrombotic product of the disclosure is provided in the dialysing solution.

In another method, the products of the disclosure are used to combat undesirable cell proliferation, as described in relation to other thrombin inhibitors in WO 01/41796. The undesirable cell proliferation is typically undesirable hyperplastic cell proliferation, for example proliferation of smooth muscle cells, especially vascular smooth muscle cells. The products of the disclosure particularly find application in the treatment of intimal hyperplasia, one component of which is proliferation of smooth muscle cells. Restenosis can be considered to be due to neointimal hyperplasia; accordingly intimal hyperplasia in the context of the disclosure includes restenosis.

25 The products of the disclosure are also contemplated for the treatment of ischemic disorders. More particularly, they may be used in the treatment (whether therapeutic or prophylactic) of an ischemic disorder in a patient having, or at risk of, non-valvular atrial fibrillation (NVAF) as described in relation to other thrombin inhibitors in WO 02/36157. Ischemic disorders are conditions whose 30 results include a restriction in blood flow to a part of the body. The term will be understood to include thrombosis and hypercoagulability in blood, tissues and/or organs. Particular uses that may be mentioned include the prevention and/or treatment of ischemic heart disease, myocardial infarction, systemic embolic events in e.g. the kidneys or spleen, and more particularly of cerebral ischemia, including cerebral thrombosis, cerebral embolism and/or cerebral ischemia associated with 35 non-cerebral thrombosis or embolism (in other words the treatment (whether therapeutic or prophylactic) of thrombotic or ischemic stroke and of transient ischemic attack), particularly in patients with, or at risk of, NVAF.

The products of the disclosure are also contemplated for the treatment of rheumatic/arthritic disorders, as described in relation to other thrombin inhibitors in WO 03/007984. Thus, the products of the disclosure may be used in the treatment of chronic arthritis, rheumatoid arthritis, osteoarthritis or ankylosing spondylitis

5

Moreover, the products of the disclosure are expected to have utility in prophylaxis of re-occlusion (i.e. thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general. Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

15

The products of the disclosure are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease. In addition to its effects on the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the disclosure may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicaemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

The compounds may also be useful in the treatment of pancreatitis.

30

The compounds described herein are further considered to be useful for inhibiting platelet procoagulant activity. The disclosure provides a method for inhibiting platelet pro-coagulant activity by administering a salt of a boronic acid described herein to a mammal at risk of, or suffering from, arterial thrombosis, particularly a human patient. Also provided is the use of such compounds for the manufacture of medicaments for inhibiting platelet procoagulant activity.

35

The use of products of the disclosure as inhibitors of platelet pro-coagulant activity is predicated on the observation that the boronic acids described herein are indicated to be effective at inhibiting arterial thrombosis as well as venous thrombosis.

Indications involving arterial thrombosis include acute coronary syndromes (especially myocardial infarction and unstable angina), cerebrovascular thrombosis and peripheral arterial occlusion and arterial thrombosis occurring as a result of atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents. Accordingly, in another aspect there is provided a

5 method of treating a disease or condition selected from this group of indications, comprising administering to a mammal, especially a human patient, a salt of the disclosure. The disclosure includes products for use in an arterial environment, e.g. a coronary stent or other arterial implant, having a coating which comprises a salt according to the disclosure.

10 The salts of the disclosure may be used prophylactically to treat an individual believed to be at risk of suffering from arterial thrombosis or a condition or disease involving arterial thrombosis or therapeutically (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

15 There is therefore included the use of selective thrombin Inhibitors (organoboronic acid salts) described herein for treatment of the above disorders by prophylaxis or therapy as well as their use in pharmaceutical formulations and the manufacture of pharmaceutical formulations.

Administration and Pharmaceutical Formulations

20 The compounds may be administered to a host, for example, in the case where the drug has anti-thrombogenic activity, to obtain an anti-thrombogenic effect. In the case of larger animals, such as humans, the compounds may be administered alone or in combination with pharmaceutically acceptable diluents, excipients or carriers. The term "pharmaceutically acceptable" includes acceptability for both human and veterinary purposes, of which acceptability for human pharmaceutical use is preferred.

25 The compounds of the disclosure may be combined and/or co-administered with any cardiovascular treatment agent. There are large numbers of cardiovascular treatment agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for use with a product of the disclosure for the prevention of cardiovascular disorders by combination drug therapy. Such agent can be one or more agents selected from, but not limited to several major categories, namely, a lipid-lowering drug, including an IBAT (ileal Na^+ /bile acid cotransporter) inhibitor, a fibrate, niacin, a statin, a CETP (cholesteryl ester transfer protein) inhibitor, and a bile acid sequestrant, an anti-oxidant, including vitamin E and probucol, a IIb/IIIa antagonist (e.g. 30 abciximab, eptifibatide, tirofiban), an aldosterone inhibitor (e.g. spirolactone and epoxymexrenone), an adenosine A2 receptor antagonist (e.g. losartan), an adenosine A3 receptor agonist, a beta-blocker, acetylsalicylic acid, a loop diuretic and an ACE (angiotensin converting enzyme) inhibitor.

The compounds of the disclosure may be combined and/or co-administered with any anti-thrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P₂T) antagonists.

5

The products of the disclosure may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

10

The compounds of the disclosure may be combined and/or co-administered with a cardioprotectant, for example an adenosine A₁ or A₃ receptor agonist.

15

There is also provided a method for treating an inflammatory disease in a patient that comprises treating the patient with a product of the disclosure and an NSAID, e.g., a COX-2 inhibitor. Such diseases include but are not limited to nephritis, systemic lupus, erythematosus, rheumatoid arthritis, glomerulonephritis, vasculitis and sarcoidosis. Accordingly, the anti-thrombotic salts of the disclosure may be combined and/or co-administered with an NSAID.

20

Typically, therefore, the compounds described herein may be administered to a host to obtain a thrombin-inhibitory effect, or in any other thrombin-inhibitory or anti-thrombotic context mentioned herein.

25

Actual dosage levels of active ingredients in the pharmaceutical compositions of this disclosure may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration (referred to herein as a "therapeutically effective amount"). The selected dosage level will depend upon the activity of the particular compound, the severity of the condition being treated and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

35

According to a further aspect there is provided a pharmaceutical formulation including a compound as described herein. The formulation may consist of the compound alone or it may contain additional components, in particular the compound may be in combination with a pharmaceutically acceptable diluent, excipient or carrier.

One class of formulations comprises parenteral formulations, these may contain suitable excipients,

for example a tonicity agent for the purpose of making the formulation substantially isotonic with the body of the subject to receive the formulation, e.g. with human plasma. The formulation may be in ready-to-use form or in a form requiring reconstitution prior to administration.

5 Intravenous formulations, in particular, will generally contain the boronic acid in a relatively soluble form, e.g. a salt, for example a base addition salt. Suitable base addition salts are, e.g. alkali metal salts, notably sodium. Also useful in intravenous formulations are sugar derivatives (esters) of the acid.

10 It is currently contemplated that, in the case of parenteral administration, for example i.v. administration, the compounds might for instance be administered in an amount of from 0.5 to 2.5mg/Kg e.g. over a maximum period of 72 hours, calculated as TRI 50F. The disclosure is not limited to administration in such quantities or regimens and includes dosages and regimens outside those described in the previous sentence.

15 Parenteral preparations can be administered by one or more routes, such as intravenous, subcutaneous, intradermal and infusion; a particular example is intravenous. A formulation disclosed herein may be administered using a syringe, injector, plunger for solid formulations, pump, or any other device recognized in the art for parenteral administration.

20 Liquid dosage forms for parenteral administration may include solutions, suspensions, liposome formulations, or emulsions in oily or aqueous vehicles. In addition to the active compounds, the liquid dosage forms may contain other compounds. Tonicity agents (for the purpose of making the formulations substantially isotonic with the subject's body, e.g. with human plasma) such as, for

25 instance, sodium chloride, sodium sulfate, dextrose, mannitol and/or glycerol may be optionally added to the parenteral formulation. A pharmaceutically acceptable buffer may be added to control pH. Thickening or viscosity agents, for instance well known cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose, hydroxyethylcellulose and hydroxypropylmethylcellulose), gelatin and/or acacia, may optionally be added to the parenteral formulation.

30 Solid dosage forms for parenteral administration may encompass solid and semi-solid forms and may include pellets, powders, granules, patches, and gels. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier. The disclosed compounds may be presented as solids in finely divided solid form, for example they

35 may be milled or micronised.

The formulations may also include antioxidants and/or preservatives. As antioxidants may be mentioned thiol derivatives (e.g. thioglycerol, cysteine, acetylcysteine, cystine, dithioerythritol, dithiothreitol, glutathione), tocopherols, butylated hydroxyanisole, butylated hydroxytoluene,

sulfurous acid salts (e.g. sodium sulfate, sodium bisulfite, acetone sodium bisulfite, sodium metabisulfite, sodium sulfite, sodium formaldehyde sulfoxylate, sodium thiosulfate) and nordihydroguaiaretic acid. Suitable preservatives may for instance be phenol, chlorobutanol, benzyl alcohol, methyl paraben, propyl paraben, benzalkonium chloride and cetylpyridinium chloride.

5

The parenteral formulations may be prepared as large volume parenterals (LVPs), e.g. larger than 100 ml, more particularly about 250 ml, of a liquid formulation of the active compound. Examples of LVPs are infusion bags. The parenteral formulations may alternatively be prepared as small volume parenterals (SVPs), e.g. about 100 ml or less of a liquid formulation of the active compound.

10 Examples of SVPs are vials with solution, vials for reconstitution, prefilled syringes for injection and dual chamber syringe devices.

15 The formulations of the disclosure include those in which the compound is an alkali metal salt, for example a lithium, sodium or potassium salt, of which sodium salts may be mentioned as particular salts. Another class of formulations contains aminosugar salts of the disclosed boronic acids, for example N-methyl-D-glucamine salts. The salts mentioned in this paragraph may be administered as solutions in water, typically containing one or more additives, for example isotonicity agent(s) and/or antioxidant(s). A suitable way to store the salts is in solid form, for example as dry powder, and to make them up into solutions for administration prior to administration.

20

25 One class of formulations disclosed herein is intravenous formulations. For intravenously administered formulations, the active compound or compounds can be present at varying concentrations, with a carrier acceptable for parenteral preparations making up the remainder. Particularly, the carrier is water, particularly pyrogen free water, or is aqueous based. Particularly, the carrier for such parenteral preparations is an aqueous solution comprising a tonicity agent, for example a sodium chloride solution.

30 By "aqueous based" is meant that formulation comprises a solvent which consists of water or of water and water-miscible organic solvent or solvents; as well as containing a compound of disclosure in dissolved form, the solvent may have dissolved therein one or more other substances, for example an antioxidant and/or an isotonicity agent. As organic co-solvents may be mentioned those water-miscible solvents commonly used in the art, for example propyleneglycol, polyethyleneglycol 300, polyethyleneglycol 400 and ethanol. Preferably, organic co-solvents are only used in cases where the active agent is not sufficiently soluble in water for a therapeutically effective amount to be provided in a single dosage form. As previously indicated, the disclosure includes formulations of alkali metal salts of the disclosed boronic acids, e.g. TRI 50F, having a solvent which consists of water.

The solubility of the active compound in the present formulations may be such that the turbidity of the formulation is lower than 50 NTU, e.g. lower than 20 NTU such as lower than 10 NTU.

5 It is desirable that parenteral formulations are administered at or near physiological pH. It is believed that administration in a formulation at a high pH (i.e., greater than 8) or at a low pH (i.e., less than 5) is undesirable. In particular, it is contemplated that the formulations would be administered at a pH of between 6.0 and 7.0 such as a pH of 6.5.

10 The parenteral formulation may be purged of air when being packaged. The parenteral formulation may be packaged in a sterile container, e.g. vial, as a solution, suspension, gel, emulsion, solid or a powder. Such formulations may be stored either in ready-to-use form or in a form requiring reconstitution prior to administration.

15 Parenteral formulations according to the disclosure may be packaged in containers. Containers may be chosen which are made of material which is non-reactive or substantially non-reactive with the parenteral formulation. Glass containers or plastics containers, e.g. plastics infusion bags, may be used. A concern of container systems is the protection they afford a solution against UV degradation. If desired, amber glass employing iron oxide or an opaque cover fitted over the container may afford the appropriate UV protection.

20 Plastics containers such as plastics infusion bags are advantageous in that they are relatively light weight and non-breakable and thus more easily stored. This is particularly the case for Large Volume parenterals.

25 The intravenous preparations may be prepared by combining the active compound or compounds with the carrier. After the formulation is mixed, it may be sterilized, for example using known methods. Once the formulation has been sterilized, it is ready to be administered or packaged, particularly in dark packaging (e.g. bottles or plastics packaging), for storage. It is envisaged, however, that the disclosed compounds might not be stored in solution but as dry solids, particularly 30 a finely divided form such as, for example, a lyophilisate, in order to prolong shelf life; this would of course apply to other parenteral formulations, not only intravenous ones.

The intravenous preparations may take the form of large volume parenterals or of small volume parenterals, as described above.

35 In a specific embodiment, the present disclosure is directed to products, particularly kits, for producing a single-dose administration unit. The products (kits) may each contain both a first container having the active compound (optionally combined with additives, for example anti-oxidant, preservative and, in some instances, tonicity agent) and a second container having the

carrier/diluent (for example water, optionally containing one or more additives, for example tonicity agent). As examples of such products may be mentioned single and multi-chambered (e.g. dual-chamber) pre-filled syringes; exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany. Such dual chamber syringes or binary syringes will have in one chamber a dry preparation including or consisting of the active compound and in another chamber a suitable carrier or diluent such as described herein. The two chambers are joined in such a way that the solid and the liquid mix to form the final solution.

5 One class of formulations disclosed herein comprises subcutaneous or intradermal formulations (for example formulations for injection) in which the active compound (or active agent combination) is formulated into a parenteral preparation that can be injected subcutaneously or intradermally. The formulation for administration will comprise the active compound and a liquid carrier.

10 The carrier utilized in a parenteral preparation that will be injected subcutaneously or intradermally may be an aqueous carrier (for example water, typically containing an additive e.g. an antioxidant and/or an isotonicity agent) or a nonaqueous carrier (again one or more additives may be incorporated). As a non-aqueous carrier for such parenteral preparations may be mentioned highly purified olive oil.

15 20 The active compound and the carrier are typically combined, for example in a mixer. After the formulation is mixed, it is preferably sterilized, such as with U.V. radiation. Once the formulation has been sterilized, it is ready to be injected or packaged for storage. It is envisaged, however, that the disclosed compounds will not be stored in liquid formulation but as dry solids, in order to prolong shelf life.

25 For making subcutaneous implants, the active compound may suitably be formulated together with one or more polymers that are gradually eroded or degraded when in use, e.g. silicone polymers, ethylene vinylacetate, polyethylene or polypropylene.

30 35 Transdermal formulations may be prepared in the form of matrices or membranes, or as fluid or viscous formulations in oil or hydrogels or as a compressed powder pellet. For transdermal patches, an adhesive which is compatible with the skin may be included, such as polyacrylate, a silicone adhesive or polyisobutylene, as well as a foil made of, e.g., polyethylene, polypropylene, ethylene vinylacetate, polyvinylchloride, polyvinylidene chloride or polyester, and a removable protective foil made from, e.g., polyester or paper coated with silicone or a fluoropolymer. For the preparation of transdermal solutions or gels, water or organic solvents or mixtures thereof may be used. Transdermal gels may furthermore contain one or more suitable gelling agents or thickeners such as silicone, tragacanth, starch or starch derivatives, cellulose or cellulose derivatives or polyacrylic acids or derivatives thereof. Transdermal formulations may also suitably contain one or more substances

that enhance absorption through the skin, such as bile salts or derivatives thereof and/or phospholipids. Transdermal formulations may be prepared according to a method disclosed in, e.g., B W Barry, "Dermatological Formulations, Percutaneous Absorption", Marcel Dekker Inc., New York--Basel, 1983, or Y W Chien, "Transdermal Controlled Systemic Medications", Marcel Dekker Inc., New York--Basel, 1987.

It is currently contemplated that, in the case of oral administration, the compounds might for instance be administered in an amount of from 0.5 to 2.5mg/Kg twice daily, calculated as TRI 50F. However, the presently described methods are not limited to administration in such quantities or 10 regimens and includes dosages and regimens outside those described in the previous sentence.

According to a further aspect there is provided an oral pharmaceutical formulation including a product as described herein, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

15 Solid dosage forms for oral administration include capsules, tablets (also called pills), powders and granules. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or one or more: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules and tablets, the dosage form may also comprise buffering agents. Solid compositions of a similar type 20 may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycol, for example.

25 Suitably, the oral formulations may contain a dissolution aid. The dissolution aid is not limited as to its identity so long as it is pharmaceutically acceptable. Examples include nonionic surface active agents, such as sucrose fatty acid esters, glycerol fatty acid esters, sorbitan fatty acid esters (e.g., sorbitan trioleate), polyethylene glycol, polyoxyethylene hydrogenated castor oil, polyoxyethylene 30 sorbitan fatty acid esters, polyoxyethylene alkyl ethers, methoxypolyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyethylene glycol fatty acid esters, polyoxyethylene alkylamines, polyoxyethylene alkyl thioethers, polyoxyethylene polyoxypropylene copolymers, polyoxyethylene glycerol fatty acid esters, pentaerythritol fatty acid esters, propylene glycol monofatty acid esters, polyoxyethylene propylene glycol monofatty acid esters, polyoxyethylene 35

sorbitol fatty acid esters, fatty acid alkylolamides, and alkylamine oxides; bile acid and salts thereof (e.g., chenodeoxycholic acid, cholic acid, deoxycholic acid, dehydrocholic acid and salts thereof, and glycine or taurine conjugate thereof); ionic surface active agents, such as sodium laurylsulfate, fatty acid soaps, alkylsulfonates, alkylphosphates, ether phosphates, fatty acid salts of basic amino acids; 5 triethanolamine soap, and alkyl quaternary ammonium salts; and amphoteric surface active agents, such as betaines and aminocarboxylic acid salts.

The active compounds may also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

10

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as water or other solvents, solubilising agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl

15

alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring and perfuming agents.

20

Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth and mixtures thereof.

The presently disclosed product may be presented as solids in finely divided solid form, for example

25

they may be micronised. Powders or finely divided solids may be encapsulated.

The active compound may be given as a single dose, in multiple doses or as a sustained release formulation.

30

The active compound, especially when a base addition salt or sugar derivative, may be administered as a drinking solution or drinking suspension.

Thus an orally administered compound may be presented as reconstitutable formulations, in particular in a form for reconstitution before administration as a liquid and often as drink, for

35

example as an effervescent tablet or in particulate form (as a powder or granules). Soluble compounds may be packaged as a powder or granules for direct dissolution in the mouth; additionally contemplated are "fast melt" or "fast dissolving" oral formulations, which dissolve or disintegrate rapidly when taken into the mouth. The formulations described in the preceding two sentences may also be regarded as reconstitutable formulations in that they are reconstituted in the

mouth, prior to the reconstituted formulation reaching the stomach. All these reconstitutable formulations avoid the delays associated with active ingredients in tablets or capsules reaching the blood, as a result of time taken for the tablet/capsule to disintegrate and for its contents to dissolve. Another potential benefit of reconstitutable formulations relates to active ingredients whose required

5 dosage is too high to be incorporated in a single tablet or capsule: the ingestion of multiple tablets or capsules is considered undesirable by patients and might create an additional risk of variation in bioavailability, and the replacement of multiple tablets or capsules with a reconstitutable formulation will avoid these particular shortcomings.

10 A common form of dosage for the oral administration of drugs is that of particulate formulations contained in dispensing containers, e.g. sachets, particularly monodose sachets. The contents of the dispensing container are usually (but not always) poured out in, for example, a glass of water or in fruit juice or in milk, for drinking by the patient. It is helpful for the particulate formulation to be reconstituted into a drink-size volume (e.g. 50-150 ml, by way of non-limiting example).

15 The dispensing container may in principle be any container which may be opened to release a single dose or a part of a dose. In many instances, a container will be a single dose or monodose container, in which the container contains the correct amount for a single administration of the formulation. Alternatively, the formulation may be presented as a divided dose, in which there is 20 provided a unit dosage which is possibly smaller than some patients require; in this latter case, the patient will take two or more unit doses in a single administration of the formulation. The dispensing container may alternatively be a metered dose container, in which a unit of formulation is metered from a reservoir of the formulation. As an alternative to a sachets, the container may be a plastics container, for example.

25 Thus, an exemplary unit dosage form for particulate formulations, e.g. for reconstitution as a drink, is the sachet. A sachet is a pouch formed by folding and / or sealing together the edges of a suitable material.

30 Particulate formulations suitable for filling into sachets may contain (but are not required to contain) diluents, flow aids, lubricants, buffering agents, granulating agents, disintegrants, solubilising agents, viscosity enhancers, sweeteners and flavours, in addition to the active ingredient. Usually, they contain anti-microbial preservations.

35 Effervescent tablets are another common oral dosage form and contain ingredients that react together in the presence of water to produce carbon dioxide. The liberation of carbon dioxide when effervescent tablets are added to water promotes their disintegration and the dissolution or dispersion of the active ingredients and other components. Effervescent tablets are usually intended to be added to water to produce a solution or dispersion for oral administration. They are usually

much bigger than other tablets because they are used for drugs whose dosage is large and they often contain relatively large amounts of flavouring agents.

5 The effervescent tablet consists of at least three components: the active ingredient; an acid; and an alkali compound (basic ingredient) constituted by a carbonate or a bicarbonate.

10 The acid and the alkali are the essential components which provide the effervescence and the disintegration of the tablet when it is contacted with water. As acidic component citric acid in anhydrous form is often used, but other edible acids like tartaric, fumaric, adipic and malic acid can be used as well. The carbonate, which represents a source of carbon dioxide which generates the effervescence, generally is a water-soluble alkaline carbonate. Sodium bicarbonate is one of the most used carbonates because it is very soluble and of low cost. Alternatively, modified sodium bicarbonate can be used, obtained by heating common sodium bicarbonate in order to convert the surface of its particles to sodium carbonate so increasing its stability.

15

Other physiologically acceptable alkaline or alkaline earth metal carbonates may be used, such as potassium or calcium (bi)carbonate, sodium carbonate, or sodium glycine carbonate.

20 Conventional excipients such as diluents, ligands, buffers, anti-microbial preservatives, sweeteners, flavours, colours, solubilisers, disintegrants, wetting agents and other excipients of common use may be added to the formulation. Effervescent tablets are convenient, attractive, easy to use premeasured dosage forms. These advantages, however, are balanced by hygroscopicity, which usually means that the tablets have to be manufactured in conditions of low relative humidity and packaged in containers that provide good protection against the ingress of water vapour.

25

"Fast melt" formulations, i.e. rapidly disintegrating or dissolving solid dose oral formulations, are described in US 5607697, WO 98/46215, WO 98/14179, US 5871781, US 5869098 and US 5464632, for example. They are also described in US 4642903, US 5188825, US 5631023 and US 5827541.

30 EXAMPLES

EXAMPLES 1 TO 4 - INTRODUCTORY REMARKS

Apparatus

35 Throughout the following procedures of Examples 1 to 4, standard laboratory glassware and, where appropriate, specialised apparatus for handling and transferring of air sensitive reagents are used.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen.

Solvents

The organic solvents used in the procedures of Examples 1 to 4 are all dry. Suitably, they are dried over sodium wire before use.

5

Dryness

In the drying procedures of Example 1 to 4, products are tested for dryness (including dryness in terms of organic solvent) by observing weight loss on drying. The following procedure was followed to determine loss on drying: a sample was placed in a vacuum drier and dried at 40°C at 100 mbar for 2 hours. Products are considered dry when the decrease in weight upon drying is less than 0.5% of the total weight of the starting material.

10 Examples 1 to 4 describe performance of the following reaction scheme and conversion of the resultant TRI 50F to sodium and calcium salts thereof:

15

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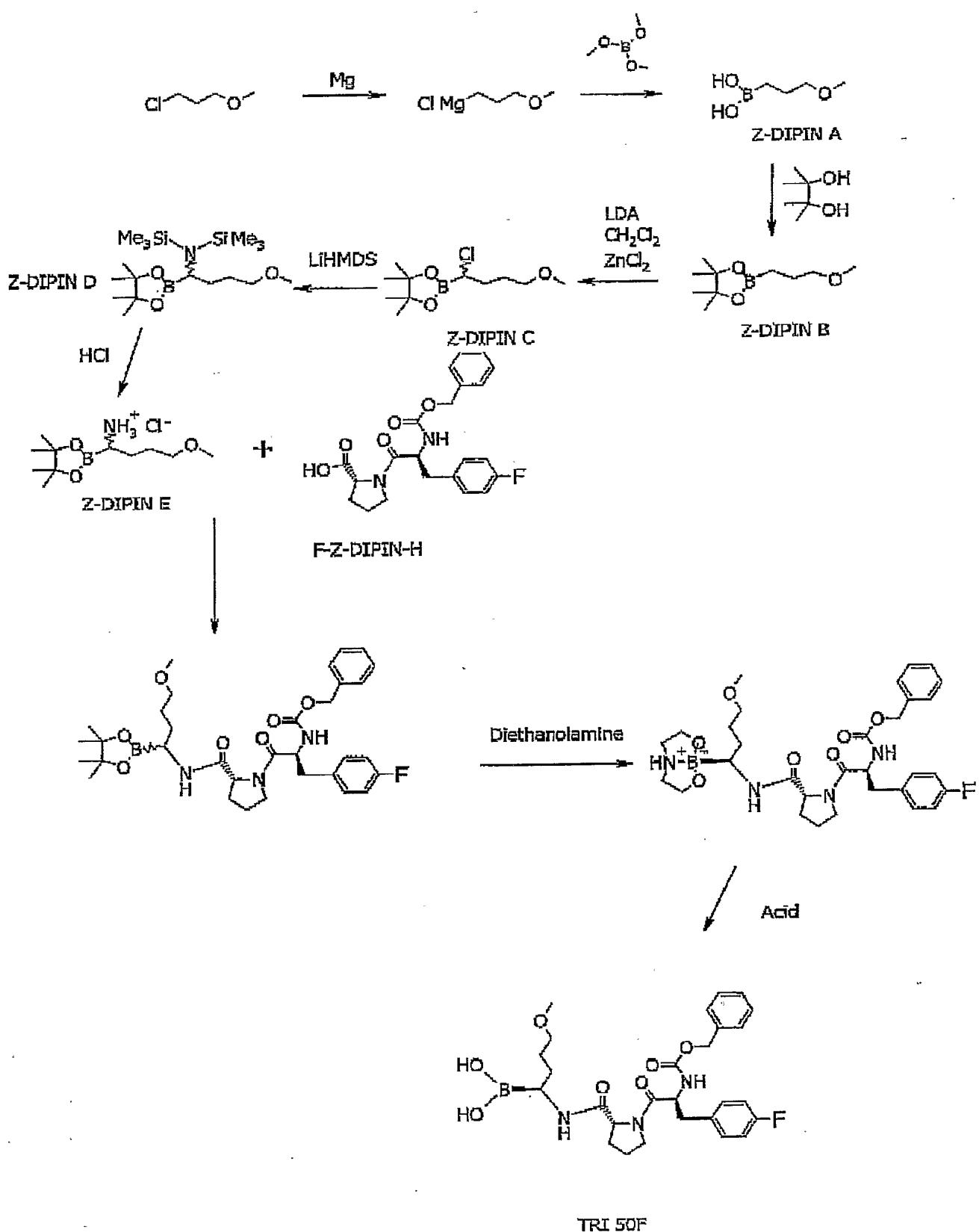
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LDA = lithium diisopropylamide

LiHMDS = lithium hexamethyldisilazane, also known as lithium bis(trimethylsilyl)amide

EXAMPLE 1 - SYNTHESIS OF TRI 5OF PINACOL ESTER**Step 1: Z-DIPIN B**

5

Procedure A

17.8 g (732.5 mmole) magnesium turnings, 0.1 g (0.4 mmole) iodine and 127 ml dry tetrahydrofuran are charged and heated to reflux. Then 15 ml of a solution of 66 g (608 mmole) 1-chloro-3-methoxypropane in 185 ml dry tetrahydrofuran are added and stirred under reflux until the

10 vigorous reaction starts. After the initial exotherm ceases, the solution of 1-chloro-3-methoxypropane is added slowly to maintain gentle reflux until all the magnesium is consumed.

After the reaction is finished, the reaction mixture is cooled to ambient temperature and slowly added to a solution of 64.4 g (620 mmole) trimethylborate in 95 ml dry tetrahydrofuran; the latter solution is cooled to below 0°C and, if it warms up during the course of the reaction, the reaction

15 mixture must be added to it sufficiently slowly to maintain the temperature of this solution below 65°C. Upon complete addition, the reaction mixture is allowed to warm to about 0°C and stirred for another 60 minutes. Then a solution of 22.4 ml sulfuric acid in 400 ml water is added slowly so as to maintain the temperature below 20°C. The layers are allowed to settle and the phases are separated. The aqueous layer is reashed three times with 200 ml tert.-butylmethylether. The

20 combined organic layers are allowed to settle and additional water separated from this solution is removed. The organic layer is dried over magnesium sulfate, filtered and evaporated to dryness. The evaporation residue is filtered from the precipitated solid and the filtrate dissolved in 175 ml toluene. 34.8 g (292 mmole) pinacol is charged to the solution followed by stirring at ambient temperature for not less than 10 hours. The solution is evaporated to dryness, dissolved in 475 ml

25 n-heptane and washed three times with 290 ml saturated aqueous solution of sodium hydrogen carbonate. The n-heptane solution is evaporated to dryness and the evaporation residue distilled and the fraction with Bp 40-50°C at 0.1-0.5 mbar recovered.

Boiling point: 40-50°C / 0.1-0.5 mbar

Yield: 40.9 g (70%) Z-DIPIN B (oil)

30

Procedure B

17.8 g (732.5 mmole) magnesium turnings, 0.1 g (0.4 mmole) iodine and 127 ml dry tetrahydrofuran are charged and heated to reflux. Then 15 ml of a solution of 66 g (608 mmole) 1-chloro-3-methoxypropane in 185 ml dry tetrahydrofuran are added and stirred under reflux until the

35 vigorous reaction starts. After the initial exotherm ceases, the solution of 1-chloro-3-methoxypropane is added slowly to maintain gentle reflux. After the reaction is finished, the reaction mixture is cooled to ambient temperature and slowly added to a solution of 64.4 g (620 mmole) trimethylborate in 95 ml dry tetrahydrofuran, maintaining the temperature of this solution below minus 65°C. Upon complete addition, the reaction mixture is allowed to warm to about 0°C

and stirred for another 60 minutes. Then a solution of 22.4 ml sulfuric acid in 400 ml water is added slowly so as to maintain the temperature below 20°C. The organic solvent is removed by distillation under vacuum. 300 ml n-heptane is charged to the aqueous solution of the evaporation residue followed by addition of 34.8 g (292 mmole) pinacol. The two-phase-mixture is stirred at ambient

5 temperature for not less than 2 hours. After allowing the layers to settle, the aqueous phase is separated. 300 ml n-heptane is charged to the aqueous solution and the two-phase-mixture is stirred at ambient temperature for not less than 2 hours. After allowing the layers to settle, the aqueous phase is separated. The organic layers are combined and washed once with 200 ml water, followed by 200 ml saturated sodium hydrogen carbonate solution and two further washes with 200

10 ml water each. The n-heptane solution is evaporated to dryness and the evaporation residue distilled and the fraction with Bp 40-50°C at 0.1-0.5 mbar recovered.

Boiling point: 40-50°C / 0.1-0.5 mbar

Yield: 40.9 g (70-85%) Z-DIPIN B (oil)

15 Step 2: Z-DIPIN C

16.6 g (164 mmole) diisopropylamine and 220 ml tetrahydrofuran are charged and cooled to -30 to -40°C. To this solution 41.8 g (163 mmole) n-butyl lithium, 25% in n-heptane is added, followed by stirring at 0 to -5°C for one hour. This freshly prepared solution of lithium diisopropylamide is

20 cooled to -30°C and then added to a solution of 27.9 g (139 mmole) Z-DIPIN B in 120 ml tetrahydrofuran and 35.5 g (418 mmole) dichloromethane at a temperature between -60 and -75°C. The solution is stirred at that temperature for half an hour followed by addition of 480 ml (240 mmole) 0.5N anhydrous Zinc(II)-chloride in tetrahydrofuran or 32.5 g (240 mmole) anhydrous solid Zinc(II)-chloride. After stirring at -65°C for one hour, the reaction mixture is allowed to warm

25 to ambient temperature and stirred for another 16-18 hours. The reaction mixture is evaporated to dryness (i.e. until solvent is removed) and followed by addition of 385 ml n-heptane. The reaction mixture is washed with 150 ml 5% sulfuric acid, with 190 ml saturated sodium hydrogen carbonate solution, and 180 ml saturated sodium chloride solution. The organic layer is dried over magnesium sulfate, filtered and evaporated to dryness (i.e. until solvent is removed). The oily residue is

30 transferred into the next step without further purification.

Yield: 19 g (55%) Z-DIPIN C

Step 3: Z-DIPIN D

35 To a solution of 23.8 g (148 mmole) hexamethyldisilazane in 400 ml tetrahydrofuran at -15°C is added 34.7 g (136 mmole) n-butyl lithium, 25% in n-heptane and stirred for one hour. The solution is cooled to -55°C followed by the addition of 30.6 g (123 mmole) Z-DIPIN C dissolved in 290 ml tetrahydrofuran and 35 ml tetrahydrofuran to this freshly prepared solution of LiHMDS. The solution is allowed to warm to ambient temperature and stirred for 12 hours. The reaction mixture is

5 evaporated to dryness, the evaporation residue dissolved in 174 ml n-heptane, washed with 170 ml water and 75 ml saturated sodium chloride solution. The organic phase is dried over magnesium sulfate, filtered and evaporated to complete dryness (i.e. until solvent is removed). The oily residue is dissolved in 100 g n-heptane. This solution is carried over into the next step without further purification.

Yield: 32.2 g (70%) Z-DIPIN D

Step 4: Z-DIPIN E

10 A solution of 26.6 g (71 mmole) Z-DIPIN D in 82.6 g n-heptane is diluted with 60 ml n-heptane and cooled to -60°C followed by introduction of 10.5 g (285 mmole) hydrogen chloride. The reaction mixture is subsequently evacuated and flushed with nitrogen, while the temperature is increased in increments of about 20°C to ambient temperature. The solvent is removed from the oily precipitate and replaced several times by 60 ml fresh n-heptane. The compound is dissolved in 60 ml

15 tetrahydrofuran (Solution A).

Step 5 TRI 50F Pinacol Ester

20 Z-DIPIN E is coupled with Z-D-4-F-Phe-Pro. The procedure described in the next paragraph is generally followed.

25 To a different flask 130 ml tetrahydrofuran, 24.5 g (61.5 mmole) Z-D-4-F-Phe-Pro and 6.22 g (61.5 mmole) N-methylmorpholine are charged and cooled to -20°C. To this solution a solution of 8.4 g (61.5 mmole) isobutylchloroformate in 20 ml tetrahydrofuran is added and stirred for 30 minutes, followed by addition of Solution A at -25°C. Upon complete addition, up to 16 ml (115 mmole) triethylamine is added to adjust the pH to 9-10, measured using a pH stick. The reaction mixture is allowed to warm to ambient temperature and stirred for 3 hours, still under nitrogen. The solvent is evaporated to dryness and the evaporation residue dissolved in 340 ml tert.-butylmethylether (t-BME). The solution of TRI 50F pinacol ester in t-BME is washed twice with 175 ml 1.5% hydrochloric acid. The combined acidic washes are given a rewash with 175 ml t-BME. The combined organic layers are washed with 175 ml water, with 175 ml saturated sodium hydrogen carbonate solution, with 175 ml 25% sodium chloride solution, dried over magnesium sulfate and filtered. This solution is carried over into the next step without further purification.

35 EXAMPLE 2 - SYNTHESIS OF TRI 50F DIETHANOLAMINE ADDUCT

The procedure described in the next paragraph is generally followed.

5 The starting material used in this Example is the solution of TRI 50F pinacol ester obtained in Example 1. The solution is carried forward to the synthesis of TRI 50d without further purification. The solution of TRI 50S pinacol ester in t-BME is evaporated to dryness and the evaporation residue dissolved in 80 ml diethylether. 1.25 equivalents of diethanolamine is added (based on HPLC determination of R,S,R TRI 50F pinacol ester) and the mixture heated at reflux for at least 10 hours, during which process the product precipitates. The suspension is cooled to 5-10°C, filtered and the filter residue washed with diethylether.

10 To improve chiral and chemical purity the wet filter cake is dissolved in 7 ml dichloromethane, cooled to 0-5°C and the product precipitated by addition of 42 ml diethylether and filtered. The isolated wet product is dried at 35°C in vacuum or at least 4 hours, until dry.

EXAMPLE 3 - PREPARATION OF SODIUM SALT OF TRI50F

15 The following procedure is generally followed.

20 2.5 mole TRI50F diethanolamine ester from Example 2 is dissolved in 10.5 L dichloromethane. 1.1 L 2% hydrochloric acid is added and the mixture is stirred for at most 30 minutes (optimally about 20 minutes) at room temperature. A precipitate forms in the organic phase. After stirring, the layers are allowed to settle and separated. The aqueous layer is rewashed twice with 2.2 L dichloromethane. The combined organic layers are washed with a solution of 625 g ammonium chloride in 2.25 L water. (The ammonium chloride buffers the pH of the aqueous extractions to be within a range of from about pH 1-2 to about pH 4-5, as strongly acidic conditions might cleave peptide bonds). The organic phase is dried over magnesium sulfate, filtered and the filtrate 25 evaporated to dryness. An assay of the free boronic acid is performed for at most 30 mins at room temperature and the amounts of the solvents and base for conversion of the acid to the salt are calculated. If 2.5 mol of the free acid is obtained, the evaporation residue is dissolved in 5 L acetonitrile followed by addition of a solution of 100 g (2.5 mole) sodium hydroxide as a 5% solution in 2.2 L water. The solution is stirred for two hours at ambient temperature (e.g. 15-30°C, optimally 30 room temperature) and then evaporated in vacuum (of ca. 10 mmHg) at a temperature not exceeding 35°C. The evaporation residue is repeatedly dissolved in 3.5 L fresh acetonitrile and evaporated to dryness to remove traces of water. If the evaporation residue is dry, it is dissolved in 3 L acetonitrile (or alternatively in 6 L THF) and slowly added to a mixture of 32 L n-heptane and 32 L diethylether. The addition is performed slowly enough to avoid lumping or sticking of the product 35 and is carried out over a period of not less than 30 minutes. The precipitated product is filtered off, washed with n-heptane and dried under vacuum at a temperature initially of about 10°C and then increasing to a limit of about 35°C, until dry.

EXAMPLE 4 - PREPARATION OF CALCIUM SALT OF TRI50F

The following procedure is generally followed.

2.5 mole TRI 50F pinacol ester from Example 2 is dissolved in 10.5 L dichloromethane. 11 L 2% hydrochloric acid is added and the mixture is stirred for at most 30 minutes (optimally about 20 minutes) at room temperature. After stirring the layers are allowed to settle and separated. The aqueous layer is reashed twice with 2.2 L dichloromethane. The combined organic layers are washed with a solution of 625 g ammonium chloride in 2.25 L water. The organic phase is dried over magnesium sulfate, filtered and the filtrate evaporated to dryness. An assay of the free boronic acid is performed and the amounts of the solvents and base for conversion of the acid to the salt are calculated. If 2.5 mol of the free acid is obtained, the evaporation residue is dissolved in 5 L acetonitrile followed by addition of a suspension of 93 g (1.25 mole) calcium hydroxide in 1 L water. The solution is stirred for two hours at ambient temperature (e.g. 15-30°C, optimally room temperature) and then evaporated under vacuum (of ca. 10 mmHg) at a temperature initially of about 10°C and then increasing to a limit of about 35°C. The evaporation residue is repeatedly dissolved in 3.5 L fresh acetonitrile and evaporated to dryness to remove traces of water. If the evaporation residue is dry, it is dissolved in 6 L tetrahydrofuran and slowly added to a mixture of 32 L n-heptane and 32 L diethylether. The addition is performed slowly enough to avoid lumping or sticking of the product and is carried out over a period of not less than 30 minutes. The precipitated product is filtered off, washed with n-heptane and dried under vacuum (of ca. 10 mmHg) at a temperature below 35°C until dry.

The procedures of Examples 1 to 4 may be scaled up and, if operated carefully, will produce highly pure salts. In the diethanolamine precipitation step it is important to use 1.25 equivalents of diethanolamine per equivalent of (R,S,R) TRI 50F pinacol ester. In the hydrolysis of the diethanolamine ester, it is important to avoid excessively long contact with the aqueous acid. Likewise the TRI 50F pinacol ester should be synthesised via the Grignard reaction to Z-DIPIN A.

EXAMPLE 5 - PREPARATION OF LITHIUM SALT OF TRI50E

30

The following procedure is generally followed.

TRI 50F (38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added LiOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water necessary with light warming for about 20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt is then dried under vacuum over silica to constant weight (72 h).

EXAMPLE 6 – PREPARATION OF ZINC SALT OF TRI 50F

5

The following procedure is generally followed.

10 The relative solubility of zinc hydroxide is such that, if the hydroxide had been used to prepare the corresponding TRI 50F salt using the procedure of Example 4, it would not have resulted in homogeneous salt formation. The following procedure is generally followed to prepare the zinc salt.

15 TRI 50F sodium salt (4.10mM) is dissolved in distilled water (100ml) at room temperature and zinc chloride in THF (4.27ml, 0.5M) is carefully added with stirring. A white precipitate that immediately formed is filtered off and washed with distilled water. This solid is dissolved in ethyl acetate and washed with distilled water (2 x 50ml). The organic solution is evacuated to dryness and the white solid produced dried over silica in a desiccator for 3 days before microanalysis.

EXAMPLE 7 – PREPARATION OF MAGNESTIUM SALT OF TRI 50F

20

The following procedure is generally followed.

25 TRI 50F (1.90mM) is dissolved in methanol (10ml) and stirred at room temperature. To this solution is added magnesium methoxide ($Mg(CH_3O)_2$) in methanol (1.05ml, 7.84 wt%). This solution is stirred for 2 hours at room temperature filtered and evacuated to 5ml. Water (25ml) is then added and the solution evacuated down to dryness to yield a white solid. This is dried over silica for 72 hours before being sent for microanalysis.

EXAMPLE 8 – RESIDUAL n-HEPTANE OF TRI 50C CALCIUM SALT

30

Salt prepared following the methods of Example 3 was tested by headspace gas chromatography. Data are shown below:

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68

Residual solvents: Headspace gas chromatography**GC Parameter:**

Column: DB-wax, 30 m, 0.32 mm ID, 5 μ
 Carrier Gas: Helium 5.0, 80 kPas
 Detector: FID, 220°C
 Injector Temp: 150°C
 Operating Conditions: 35°C/7 min; 10°C/ min up to 80°C/2 min; 40°C up to 180°C/2 min
 Injection volume: 1 ml
 Split: On

Headspace Parameter:

Oven temperature: 70°C
 Needle temperature: 90°C
 Transfer temperature: 100°C
 Other parameters: temper time: 15 min, GC-cycle time: 28 min,
 injection time: 0.03 min, duration: 0.4 min

Calibration Standards: sample weight/dilution

standard	weight (mg)	volume (ml)	concentration (mg/ml)	area (average, n=3)
n-heptane	103.12	100	1.0312	2757.74756
sample no.	weight (mg)	volume (ml)	concentration (mg/ml)	
1	100.84	5	20.17	
2	99.12	5	19.82	
3	100.03	5	20.01	

n-heptane	sample	concentration (mg/ml)	content (%)
	1	0.0010	0.0048
	2	0.0009	0.0044
	3	0.0010	0.0050
		0.00095	0.005

5 EXAMPLE 9- HPLC CHROMATOGRAMS

TRI 50c monosodium salt made following the method of Example 3 and TRI 50c hemicalcium salt made following the method of Example 4 were analysed by HPLC chromatography.

10 2. Method**1.1 Equipment and software**

15	Autosampler	Waters Alliance 2795
	Pump	Waters Alliance 2795
	Column oven	Waters Alliance 2795
	Detection	Waters 2996 diode array, MS-ZQ single quad
	Software version	Waters Millennium 4.0

1.2 Stationary phase

5 Analytical Column ID S-71
 Material Xterra™ MS C₁₈, 5 µm
 Supplier Waters, Eschborn, Germany
 Dimension 150 mm x 2.1 mm (length, ID)
 Pre-column ID no pre-column

10 Xterra MS C₁₈, 5 µm is a column packing material supplied by Waters Corporation, 34 Maple Street, Milford, MA 01757, US and local offices, as in years 2002/2003. It comprises hybrid organic/inorganic particles, consisting of spherical particles of 5 µm size, 125 Å pore size and 15.5% carbon load.

1.3 Mobile Phase

15 Aqueous phase: A: H₂O + 0.1% HCOOH
 Organic phase: C: ACN

H₂O = H₂O by Ultra Clear water purification system

ACN = gradient grade acetonitrile

20

Gradient conditions

time [min]	A%	C%	flow [mL/min]	gradient shape
0.0	90.0	10.0	0.5	
27.00	10.0	90.0	0.5	linear
27.10	90.0	10.0	0.5	linear
30.00	90.0	10.0	0.5	linear

1.4 Instrumental Parameters

25 Flow 0.5 mL·min⁻¹
 Temperature 40 ± 5° C
 HPLC control Waters Millennium Release 4.0
 Calculation Waters Millennium 4.0

2. Parameters

30

2.1 Wavelength/Retention time/Response factors

Table: retention and detection parameter (k' F; 0.5 mL/min, t_0 = 0.9 mL/min)

Substance	RetTime [min]	λ [nm]	m/z	response factor [area/ μ g]	Reciprocal Response factor
TRI 50c	11.68	258	508.33	660	1
Benzyl alcohol	3.862	258	n.d.	1960	0.337
Benzaldehyde	6.13	258	n.d.	79939	0.0083
Benzoic acid	5.52	258	n.d.	5967	0.111
Impurity I	11.18	258	396.17	886	0.745
Impurity II	13.39	258	482.22	552	1.196

5 2.2 Linearity

Linearity Range 4000 - 10 μ g/mL (detection UV 258 nm)

Table Linearity data UV 258nm

calibration solution	area [μ AU's]	target conc. [μ g/mL]	conc. found ¹ [μ g/mL]
Tri 50c	5353	10	20.44
Tri 50c	5301	10	20.37
Tri 50c	65809	100	113.35
Tri 50c	66365	100	114.17
Tri 50c	172019	250	270.43
Tri 50c	162587	250	256.48
Tri 50c	339503	500	518.13
Tri 50c	326912	500	499.51
Tri 50c	659257	1000	991.02
Tri 50c	647495	1000	973.63
Tri 50c	1322371	2000	1971.72
Tri 50c	1305196	2000	1946.32
Tri 50c	2724410	4000	4045.24

¹ recalculated with linear equation

10 Linear equation parameters:

Y = 6.75e+002 X - 8.45e+003

r = 0.99975

r² = 0.99950

15 Linearity Range 10 - 0,10 μ g/mL (detection SIR m/z 508,33)

Table: Linearity data SIR 508.33

calibration solution	mean area [μ AU's]	target conc. [μ g/mL]	conc. found ¹ [μ g/mL]
Tri 50c	2188860	0.01	0.022
Tri 50c	2702839	0.01	0.045
Tri 50c	3817226	0.1	0.094
Tri 50c	3833799	0.1	0.095
Tri 50c	23153550	1	0.947
Tri 50c	24646892	1	1.013
Tri 50c	223007852	10	9.765

Tri 50c	233753043	10	10.239
¹ recalculated with linear equation			

Equation parameter

$$Y = 2.27e+007 X + 1.69e+006$$

$$r = 0.99958$$

$$S r^2 = 0.99916$$

2.3 Quantitation limit

The quantitation limit was determined using the signal to noise ratio criterion S/N > 19,
UV 258 nm: 10 $\mu\text{g/mL}$

10 M/z 508.3: 0.1 $\mu\text{g/mL}$

2.4 Precision

Injection	Target concentration [$\mu\text{g/mL}$]	Area	Amount [$\mu\text{g/mL}$]	Retention time [min]
1	250	165805	261.24	11.690
2	250	168644	265.44	11.662
3	250	167858	264.27	11.686
4	250	166947	262.93	11.692
5	250	166925	262.89	11.679
6	250	166294	261.96	11.696
Mean		167079	263.12	11.684
Std. Dev.		1033	1.528	0.01
% RSD		0.6	0.6	0.1

15 2.5 Robustness

Table: robustness data; Standard 250 $\mu\text{g/mL}$ aqueous solution (containing < 1% ACN)

calibration solution	temp./time [$^{\circ}\text{C}/\text{h}$]	area [$\mu\text{AU}'s$]	recovery [%]
250 $\mu\text{g/mL}$ Tri50c	-	172020	-
250 $\mu\text{g/mL}$ Tri50c	4 $^{\circ}\text{C}$, 16h	166294	96.67
2.5 $\mu\text{g/mL}$ TR150c	-	88034891	-
2.5 $\mu\text{g/mL}$ TR150c	37 $^{\circ}\text{C}$, 4h	88833175	100.9

References

1. ICH HARMONISED TRIPARTITE GUIDELINE. TEXT ON VALIDATION OF ANALYTICAL PROCEDURES Recommended for Adoption at Step 4 of the ICH Process on 27 October 1994 by the ICH Steering Committee
2. FDA Reviewer Guidance. Validation of chromatographic methods. Center for Drug Evaluation and Research. Nov. 1994
3. USP 23. <621> Chromatography

4. L. Huber. Validation of analytical Methods. LC-GC International Feb. 1998

5. Handbuch Validierung in der Analytik. Dr. Stavros Kromidas (Ed.) Wiley-VCH Verlag. 2000. ISBN 3-527-29811-8

5

5. Results

3.1 Sample Name: TRI 50c monosodium salt

Injection volume: 10µL

10	Name	Ret Time (Min)	Area %	Area [µAU's]	Peak Height µAU
	TRI 50c	12.136	100.0000	604.27228	32.05369

3.2 Sample Name: TRI 50c hemicalcium salt

Injection volume: 10µL

15	Name	Ret Time (Min)	Area %	Area [µAU's]	Peak Height µAU
	TRI 50c	12.126	100.0000	597.11279	32.29640

20 The disclosed methods have been used to obtain salts substantially free of C-B bond degradation products, in particular salts containing no such products in an amount detectable by HPLC, specifically the method of Example 43. The disclosed methods have been used to obtain salts substantially free of Impurity I, in particular containing no Impurity I in an amount detectable by HPLC, specifically by the method of Example 43. The disclosed methods have been used to obtain salts substantially free of Impurity IV, in particular containing no Impurity IV in an amount detectable by HPLC, specifically by the method of Example 43.

25

25 salts substantially free of Impurity IV, in particular containing no Impurity IV in an amount detectable by HPLC, specifically by the method of Example 43.

EXAMPLE 10 - DETERMINATION OF DIASTEREOMERIC EXCESS

30 TRI 50b, crude, contains three chiral centres. Two of them are fixed by the use of enantiomerically pure amino acids ((R)-Phe and (S)-Pro). The third one is formed during the synthesis. The favoured epimer is the desired TRI 50b, Isomer I (R,S,R-TRI 50b). Both epimers of TRI 50b are clearly baseline separated by the HPLC method, thus allowing determination of the diasteromeric excess (de) of TRI 50b.

35

TRI 50d is not stable under the conditions applied for HPLC purity determination, but decomposes rapidly on sample preparation to TRI 50c, so that TRI 50d and TRI 50c show the same HPLC traces.

The two isomers of TRI 50c are not baseline separated in HPLC, but both isomers are clearly visible.

40 This becomes obvious, when TRI 50b, crude (mixture of both isomers) is converted with

phenylboronic acid to TRI 50c, crude. Both isomers of TRI 50c are observed in HPLC nearly at the same relation as before in TRI 50b, crude.

Upon synthesis of TRI 50d from TRI 50b, crude, only one diastereoisomer is precipitated. In this 5 case HPLC shows only one peak for TRI 50c, where a very small fronting is observed. Precipitation from dichloromethane/diethylether removes the fronting efficiently. The level of removal of Isomer II cannot be quantified by this HPLC method. Therefore samples before reprecipitation and after one and two reprecipitations were esterified with pinacol and the resulting samples of TRI 50b 10 analysed by HPLC. Thus a de of 95.4% was determined for the crude sample. The reprecipitated sample resulted in a de of 99.0% and finally the sample that was reprecipitated twice showed a de of 99.5%.

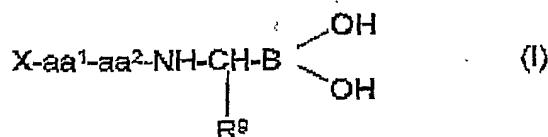
These results clearly show the preferred precipitation of Isomer I, whereas Isomer II remains in solution.

15 It will be appreciated from the foregoing that the disclosure provides boronic acid salts useful for pharmaceutical purposes and which feature one or more of the following attributes: (1) improved hydrolytic stability; (2) improved stability against deboronation; and (3), in any event, not suggested by the prior art.

20 The selection of active ingredient for a pharmaceutical composition is a complex task, which requires consideration not only of biological properties (including bioavailability) but also of physicochemical properties desirable for processing, formulation and storage. Bioavailability itself is dependent on various factors, often including in vivo stability, solvation properties and absorption properties, each 25 in turn potentially dependent on multiple physical, chemical and/or biological behaviours.

The present disclosure includes the subject matter of the following paragraphs:

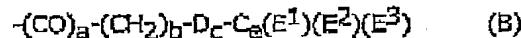
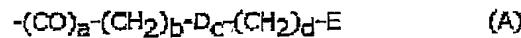
1. A compound selected from boronic acids of formula (I), and salts, prodrugs and prodrug 30 salts thereof formula (I):



wherein

35 X is H (to form NH₂) or an amino-protecting group;

aa¹ is an amino acid residue having a side chain selected from formula (A) and (B):



5

wherein

a is 0 or 1;

e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 5 or, as the case may 10 be, (b+e) is from 1 to 5;

c is 0 or 1;

D is 0 or S;

E is a saturated or unsaturated cyclic hydrocarbyl group which normally contains up to 14 members; and

15 E¹, E² and E³ are each independently selected from the group consisting of 5-6 membered saturated or unsaturated hydrocarbyl rings, or one of E¹, E² and E³ is hydrogen and the other two are a said hydrocarbyl ring,

20 E, E¹, E² and E³ optionally being halogenated when saturated and mandatorily being halogenated when unsaturated, a particular halogen being fluorine;

aa² is a residue of an amino acid which binds to the thrombin S2 subsite;; and

25 R⁹ is a straight chain alkyl group interrupted by one or more ether linkages (e.g. 1 or 2) and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R⁹ is $-(\text{CH}_2)_m-\text{W}$ where m is 2, 3, 4 or 5 (e.g. 4) and W is -OH or halogen (F, Cl, Br or I). R⁹ is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

30 2. A compound of paragraph 1 wherein R⁹ is an alkoxyalkyl group.

3. A compound of paragraph 1 or paragraph 2 wherein R⁹ is methoxypropyl.

4. A compound of any of paragraphs 1 to 3 wherein a and c are both 0 and (a+b+c+d) and (a+b+c+e) are 1, 2 or 3,

35

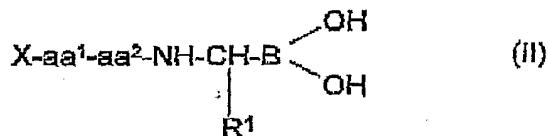
5. A compound of any of paragraphs 1 to 4 wherein E, E¹, E² and E³ are each independently phenyl fluorinated at at least the 4-position or optionally fluorinated cyclohexyl, one E¹, E² and E³ optionally being H.

5 6. A compound of paragraph 4 or paragraph 5 wherein aa¹ is of R configuration, aa² is of S configuration, and the fragment, -NHCH(R⁹)-B(OH) is of R configuration.

7. A compound of any of paragraphs 1 to 6 wherein the boronic acid has a Ki for thrombin of about 100 nM or less.

10 8. A compound of paragraph 7 wherein the boronic acid has a Ki for thrombin of about 20 nM or less.

9. A compound in pharmaceutical dosage selected from boronic acids of formula (II), and salts, 15 prodrugs and prodrug salts thereof:



where:

20 X is H (to form NH₂) or an amino-protecting group;

aa¹ is an amino acid having a side chain selected from:

25 -CH₂-E (A')
 -C(E¹)(E²)(E³) (B')

where

E is a saturated or unsaturated cyclic hydrocarbyl group which normally contains up to 14 members; and

30 E¹, E² and E³ are each independently selected from the group consisting of 5-6 membered saturated or unsaturated hydrocarbyl rings, or one of E¹, E² and E³ is hydrogen and the other two are a said hydrocarbyl ring.

E, E¹, E² and E³ optionally being halogenated when saturated and mandatorily being halogenated when unsaturated, a particular halogen being fluorine;

aa² is an imino acid having from 4 to 6 ring members;

5

R¹ is a group of the formula -(CH₂)_s-Z, where s is 2, 3 or 4 and Z is -OH, -OMe, -OEt or halogen (F, Cl, Br or I).

10. A compound of paragraph 9 wherein E, E¹, E² and E³ are each independently phenyl fluorinated at at least the 4-position or optionally fluorinated cyclohexyl, one of E¹, E² and E³ optionally being H.

11. A compound of paragraph 9 wherein aa¹ is selected from di(4-F)-Dpa, 4-F-Phe, Dcha and Cha.

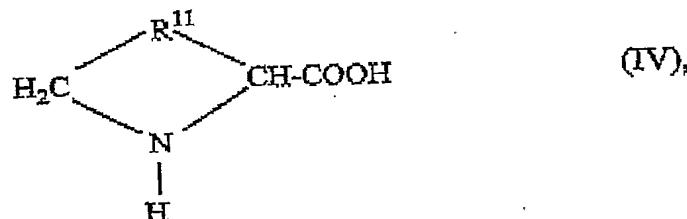
15

12. A compound of any of paragraphs 9 to 11 wherein aa¹ is of R-configuration.

13. A compound of paragraph 9 wherein aa¹ is (R)-4-F-Phe or (R)-di(4-F)-Dpa.

20. 14. A compound of paragraph 9 wherein aa¹ is (R)-4-F-Phe.

15. A compound of any of paragraphs 9 to 14 wherein aa² is a residue of an imino acid of formula (IV)



25. where R¹¹ is -CH₂-, -CH₂-CH₂-, -CH=CH-, -S-CH₂-, -S-C(CH₃)₂- or -CH₂-CH₂-CH₂-, which group, when the ring is 5- or 6-membered, is optionally substituted at one or more -CH₂- groups by from 1 to 3 C₁-C₃ alkyl groups.

16. A compound of paragraph 15 wherein aa² is of S-configuration.

30

17. A compound of paragraph 15 wherein aa² is an (S)-proline residue.

18. A compound of paragraph 9, wherein aa¹-aa² is a residue of (R)-Phe-(S)-Pro.

5 19. A compound of any of paragraphs 9 to 18 wherein R¹ is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl.

20. A compound of any of paragraphs 9 to 18 wherein R¹ is 3-methoxypropyl.

10 21. A compound of any of paragraphs 9 to 20 where X is R⁶-(CH₂)_p-C(O)-, R⁶-(CH₂)_p-S(O)₂-,
R⁶-(CH₂)_p-NH-C(O)- or R⁶-(CH₂)_p-O-C(O)- wherein p is 0, 1, 2, 3, 4, 5 or 6 and R⁶ is H or a 5 to
13-membered cyclic group optionally substituted by one or more (e.g. 1, 2, 3, 4 or 5) halogens (e.g.
F), for example at least at the 4-position, and/or by 1, 2 or 3 substituents selected from amino,
nitro, hydroxy, a C₅-C₆ cyclic group, C₁-C₄ alkyl and C₁-C₄ alkyl containing, and/or linked to the
15 cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a
substituent selected from halogen, amino, nitro, hydroxy and a C₅-C₆ cyclic group.

22. A compound of paragraph 21 wherein said 5 to 13-membered cyclic group is aromatic or
heteroaromatic.

20 23. A compound of paragraph 22 wherein said 5 to 13-membered cyclic group is phenyl or a 6-
membered heteroaromatic group.

24. A compound of any of paragraphs 9 to 20 wherein X is R⁶-(CH₂)_p-C(O)- or R⁶-(CH₂)_p-O-
25 C(O)- and p is 0, 1 or 2.

25. A compound of any of paragraphs 9 to 20 wherein X is benzyloxycarbonyl.

26. A compound of paragraph 9 wherein the boronic acid is of formula (VIII):

30 X-(R)-4-F-Phe-(S)-Pro-(R)-Mpg-B(OH)₂ (VIII)

32. A compound of any of paragraph 1 to 31 wherein said at least one substituent is selected
from halo, hydroxy, amino, amidino, guanidino, nitro, carboxyl (whether as free acid or salt),
35 esterified carboxyl (e.g. having C₁-C₄ alkyl or alkoxyalkyl as the ester-forming group, for example).

40. A compound of any of paragraphs 1 to 39 which comprises the free boronic acid.

5 41. A compound of any of paragraphs 1 to 39 which comprises a prodrug of the boronic acid.

42. A compound of any of paragraphs 1 to 39 which comprises an ester of the boronic acid.

10 43. A compound of paragraph 42 wherein the ester is with a diol.

44. A compound of paragraph 43 wherein the diol is pinacol, pinanediol, diethanolamine, neopentylglycol, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol or 1,2-dicyclohexylethanediol.

15 45. A compound of paragraph 43 wherein the diol is a sugar.

46. A compound of paragraph 45 wherein the sugar is a monosaccharide or a disaccharide.

20 47. A compound of paragraph 45 or 46 wherein the sugar is a reduced sugar.

48. A compound of paragraph 47 wherein the sugar is mannitol or sorbitol.

49. A compound of any of paragraphs 1 to 39 which comprises a base addition salt of the
25 boronic acid.

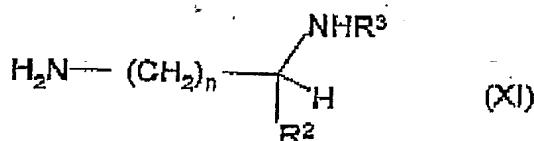
50. A compound of paragraph 49 wherein the salt comprises boronate ions derived from the boronic acid and monovalent counter-ions.

30 51. A compound of paragraph 49 or paragraph 50 which comprises a salt of the peptide boronic acid with an alkali metal or a strongly basic organic nitrogen-containing compound.

52. A compound of paragraph 51 wherein the strongly basic organic nitrogen-containing compound is a guanidine, a guanidine analogue or an amine.

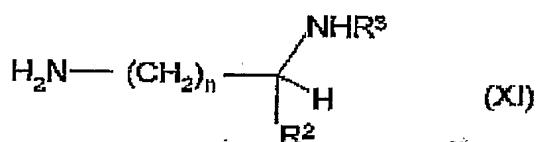
35 53. A compound of any of paragraphs 49 to 52 wherein the salt comprises a salt of the boronic acid with a metal.

54. A compound of paragraph 49 wherein the salt comprises a salt of the boronic acid with an alkali metal, an aminosugar, a guanidine or an amine of formula (XI):



5 where n is from 1 to 6, R² is H, carboxylate or derivatised carboxylate, R³ is H, C₁-C₄ alkyl or a residue of a natural or unnatural amino acid.

55. A compound of paragraph 49 wherein the salt comprises a salt of the boronic acid with a guanidine or with an amine of formula (XI):



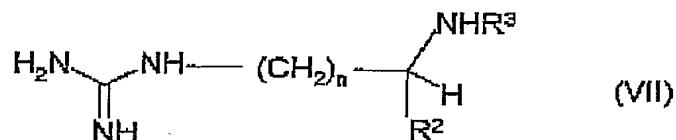
10 where n is from 1 to 6, R² is H, carboxylate or derivatised carboxylate, R³ is H, C₁-C₄ alkyl or a residue of a natural or unnatural amino acid.

56. A compound of paragraph 55 which comprises a guanidine salt of the boronic acid.

15 57. A compound of paragraph 56 which comprises a salt of the boronic acid with L-arginine or an L-arginine analogue.

20 58. A compound of paragraph 57 wherein the L-arginine analogue is D-arginine, or the D- or L-isomers of homoarginine, agmatine [(4-aminobutyl) guanidine], NG-nitro-L-arginine methyl ester, or a 2-amino pyrimidines.

59. A compound of paragraph 56 which comprises a salt of the boronic acid with a guanidine of formula (VII)



25 where n is from 1 to 6, R² is H, carboxylate or derivatised carboxylate, R³ is H, C₁-C₄ alkyl or a residue of a natural or unnatural amino acid.

60. A compound of paragraph 59, wherein n is 2, 3 or 4.

61. A compound of paragraph 59 or paragraph 60 where the derivatised carboxylate forms a C₁-C₄ alkyl ester or amide.
62. A compound of any of paragraphs 59 to 61 wherein the compound of formula (VII) is of L-configuration.
63. A compound of paragraph 56 which comprises an L-arginine salt of the peptide boronic acid.
64. A compound of paragraph 55 which comprises a salt of the boronic acid with an amine of formula (IX).
65. A compound of paragraph 64, wherein n is 2, 3 or 4.
66. A compound of paragraph 64 or paragraph 65 where the derivatised carboxylate forms a C₁-C₄ alkyl ester or amide.
67. A compound of any of paragraphs 64 to 66 wherein the amine of formula (IX) is of L-configuration.
68. A compound of paragraph 64 which comprises an L-lysine salt of the boronic acid.
69. A compound of paragraph 49 wherein the salt comprises an alkali metal salt of the boronic acid.
70. A compound of paragraph 69 wherein the alkali metal is potassium.
71. A compound of paragraph 69 wherein the alkali metal is sodium.
72. A compound of paragraph 69 wherein the alkali metal is lithium.
73. A compound of paragraph 49 wherein the salt comprises an aminosugar salt of the boronic acid.
74. A compound of paragraph 73 wherein the aminosugar is a ring-opened sugar.
75. A compound of paragraph 74 wherein the aminosugar is a glucamine.
76. A compound of paragraph 73 wherein the aminosugar is a cyclic aminosugar.

77. A compound of any of paragraphs 73 to 76 wherein the aminosugar is N-unsubstituted.

78. A compound of any of paragraphs 73 to 76 wherein the aminosugar is N-substituted by one or two substituents.

5

79. A compound of paragraph 78 wherein the or each substituent is a hydrocarbyl group.

80. A compound of paragraph 78 wherein the or each substituent is selected from the group consisting of alkyl and aryl moieties.

10

81. A compound of paragraph 80 wherein the or each substituent is selected from the group consisting of C₁, C₂, C₃, C₄, C₅, C₆, C₇ and C₈ alkyl groups

82. A compound of any of paragraphs 78 to 81 wherein there is a single N-substituent.

15

83. A compound of paragraph 73 wherein the glucamine is N-methyl-D-glucamine.

84. A compound of any of paragraphs 49 to 83 which comprises boronate ions derived from the peptide boronic acid and has a stoichiometry consistent with the boronate ions carrying a single negative charge.

20

85. A compound of any of paragraphs 49 to 83 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

25

86. A compound of any of paragraphs 49 to 85 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counter-ion and wherein the salt consists essentially of a salt having a single type of counter-ion.

30

87. A product for use as a pharmaceutical, comprising a compound of any of paragraphs 1 to 86.

88. A pharmaceutical formulation comprising a compound of any of paragraphs 1 to 86.

35

89. A pharmaceutical formulation of paragraph 88 which is adapted for intravenous administration.

90. A pharmaceutical formulation of paragraph 88 which is adapted for oral administration.

91. A method of inhibiting thrombin in the treatment of disease comprising administering to a mammal a therapeutically effective amount of a compound of any of paragraph 1 to 86.

92. The use of a compound of any of paragraph 1 to 86 for the manufacture of a medicament for treating thrombosis.

93. A method of treating venous and/or arterial thrombosis by prophylaxis or therapy, comprising parenterally administering to a mammal suffering from, or at risk of suffering from, arterial thrombosis a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

94. A method of paragraph 93 wherein the disease is an acute coronary syndrome.

95. A method of paragraph 93 wherein the disease is acute myocardial infarction.

96. A method of paragraph 93 wherein the disease is a venous thromboembolic event, selected from the group consisting of deep vein thrombosis and pulmonary embolism.

97. A method for preventing thrombosis in a haemodialysis circuit of a patient, comprising parenterally administering to the patient a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

98. A method for preventing a thrombotic event in a patient with end stage renal disease, comprising parenterally administering to the patient a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

99. A method for preventing venous thromboembolic events in a patient receiving chemotherapy through an indwelling catheter, comprising administering to the patient a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

100. A method for preventing thromboembolic events in a patient undergoing a lower limb arterial reconstructive procedure, comprising parenterally administering to the patient a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

101. A method of inhibiting platelet procoagulant activity, comprising parenterally administering to a mammal at risk of, or suffering from, arterial thrombosis a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

102. A method of paragraph 101 wherein the disease is an acute coronary syndrome.

103. A method of treating by way of therapy or prophylaxis an arterial disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, comprising parenterally administering to a mammal a therapeutically effective amount of a product selected from the compounds defined any of paragraphs 1 to 86.

104. A method of paragraph 103 wherein the disease is an acute coronary syndrome.

105. The use of a compounds defined any of paragraphs 1 to 86 for the manufacture of a parenteral medicament for a treatment recited in any of paragraphs 99 to 104.

106. A pharmaceutical formulation comprising a combination of (i) a compound defined any of paragraphs 1 to 86 and (ii) a further pharmaceutically active agent.

107. A pharmaceutical formulation comprising a combination of (i) a compound defined any of paragraphs 1 to 86 and (ii) another cardiovascular treatment agent.

108. A formulation of paragraph 107 wherein the other cardiovascular treatment agent comprises a lipid-lowering drug, a fibrate, niacin, a statin, a CETP inhibitor, a bile acid sequestrant, an antioxidant, a IIb/IIIa antagonist, an aldosterone inhibitor, an A2 antagonist, an A3 agonist, a beta-blocker, acetylsalicylic acid, a loop diuretic, an ace inhibitor, an antithrombotic agent with a different mechanism of action, an antiplatelet agent, a thromboxane receptor and/or synthetase inhibitor, a fibrinogen receptor antagonist, a prostacyclin mimetic, a phosphodiesterase inhibitor, an ADP-receptor (P₂T) antagonist, a thrombolytic, a cardioprotectant or a COX-2 inhibitor.

109. The use of a compound defined any of paragraphs 1 to 86 for the manufacture of a parenteral medicament for treating, for example preventing, a cardiovascular disorder in co-administration with another cardiovascular treatment agent.

110. A medicament comprising a boronic acid which is a selective thrombin inhibitor and has a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked through a peptide linkage to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites, the hydrophobic moiety comprising a fluorinated aromatic ring in its S3-binding part, or a salt, prodrug or prodrug salt thereof.

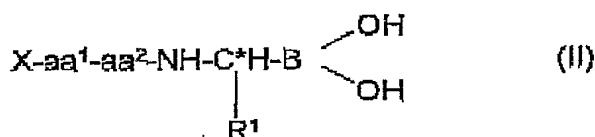
111. A medicament of paragraph 111 wherein the boronic acid has a Ki for thrombin of about 100 nM or less.

112. A medicament of paragraph 111 wherein the boronic acid has a K_i for thrombin of about 20 nM or less.

5 113. A medicament comprising a sodium salt of $\text{Cbz}-(\text{R})-\text{4-F-Phe}-(\text{S})-\text{Pro}-(\text{R})-\text{Mpg-B(OH)}_2$.

114. A pharmaceutical product comprising a sealed container containing in the form of a finely divided solid, ready for reconstitution to form a liquid formulation, a therapeutically effective amount of a boronic acid formula (II) or a salt, prodrug or prodrug salt thereof:

10



where:

X is H (to form NH_2) or an amino-protecting group;

15

aa^1 is as defined in paragraph 9;

aa^2 is an imino acid of S-configuration having from 4 to 6 ring members;

20

C^* is a chiral centre of R-configuration;

and

R^1 is a group of the formula $-(\text{CH}_2)_s-\text{Z}$, where s is 2, 3 or 4 and Z is $-\text{OH}$, $-\text{OMe}$, $-\text{OEt}$ or halogen

25 (F, Cl, Br or I).

115. The product of paragraph 114 wherein:

X is $\text{R}^6-(\text{CH}_2)_p-\text{O(O)}$, $\text{R}^6-(\text{CH}_2)_p-\text{S(O)}_2$, $\text{R}^6-(\text{CH}_2)_p-\text{NH-C(O)}$ or $\text{R}^6-(\text{CH}_2)_p-\text{O-C(O)}$ wherein p is 0,

30 1, 2, 3, 4, 5 or 6 and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by 1, 2 or 3 substituents selected from halogen, amino, nitro, hydroxy, a $\text{C}_5\text{-C}_6$ cyclic group, $\text{C}_1\text{-C}_4$ alkyl and $\text{C}_1\text{-C}_4$ alkyl containing, and/or linked to the cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a $\text{C}_5\text{-C}_6$ cyclic group;

aa¹ is selected from (R)-4-F-Phe, (R)-4-F-Dpa, (R)-Cha and (R)-Dcha;

aa² is Pro; and

5

R¹ is 2-ethoxyethyl or 3-methoxypropyl.

117. A pharmaceutical formulation adapted for oral or intravenous administration, whether directly or after combining with a liquid, and comprising

10

a) a first species selected from (a) boronic acids of formula (I) as defined in paragraph 1, (b) boronate anions thereof, and (c) any equilibrium form of the foregoing (e.g. an anhydride; and

15

(b) a second species selected from the group consisting of pharmaceutically acceptable cations having a valency n,

20 wherein the formulation has an observed stoichiometry of first to second species essentially consistent with a notional stoichiometry of 1:1 except where the second species is a metal ion having a valency of greater than 1, in which case the observed stoichiometry is essentially consistent with a notional stoichiometry of n:1.

25 118. The formulation of paragraph 117 which has the characteristic that, after the formulation if not in an aqueous carrier is placed in one, it has a Ki for thrombin of about 20 nM or less.

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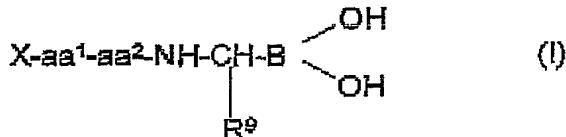
119. The formulation of any of paragraphs 88, 89, 106 to 108, 117 or 118 or a medicament of any of paragraphs 110 to 113 which is in unit dosage form.

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CLAIMS

1. A compound selected from boronic acids of formula (I), and salts, prodrugs and prodrug salts thereof:

5



wherein

X is H (to form NH_2) or an amino-protecting group;

10

aa¹ is an amino acid residue having a side chain selected from formula (A) and (B):

-(CO)_a-(CH₂)_b-D_c-(CH₂)_d-E (A)

-(CO)_a-(CH₂)_b-D_c-C_e(E¹)(E²)(E³) (B)

15

wherein

a is 0 or 1;

e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 5 or, as the case may be, (b+e) is from 1 to 5;

20

c is 0 or 1;

D is O or S;

E is a saturated or unsaturated cyclic hydrocarbyl group which normally contains up to 14 members; and

25

E¹, E² and E³ are each independently selected from the group consisting of 5-6 membered saturated or unsaturated hydrocarbyl rings, or one of E¹, E² and E³ is hydrogen and the other two are a said hydrocarbyl ring,

30

E, E¹, E² and E³ optionally being halogenated when saturated and mandatorily being halogenated when unsaturated, a particular halogen being fluorine;

aa² is a residue of an amino acid which binds to the thrombin S2 subsite; and

R^9 is a straight chain alkyl group interrupted by one or more ether linkages and in which the total number of oxygen and carbon atoms is 3, 4, 5 or 6 (e.g. 5) or R^9 is $-(CH_2)_m-W$ where m is from 2, 3, 4 or 5 (e.g. 4) and W is $-OH$ or halogen (F, Cl, Br or I). As examples of straight chain alkyl interrupted by one or more ether linkages (-O-) may be mentioned alkoxyalkyl (one interruption) 5 and alkoxyalkoxyalkyl (two interruptions). R^9 is an alkoxyalkyl group in one subset of compounds, e.g. alkoxyalkyl containing 4 carbon atoms.

2. A compound of claim 1 wherein R^9 is an alkoxyalkyl group.

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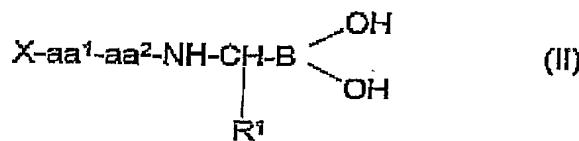
4. A compound of any of claims 1 to 3 wherein a and c are both 0 and $(a+b+c+d)$ and $(a+b+c+e)$ are 1, 2 and 3.

15 5. A compound of claim 4 wherein aa^1 is of (R)-configuration, aa^2 is of (S)-configuration, and the fragment $-NHCH(R^9)-B(OH)$ is of (R)-configuration.

7. A compound of any of claims 1 to 6 whereinsaid at least one substituent comprises halogen, hydroxy, amino, nitro, carboxyl or esterified carboxyl.

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9. A compound selected from boronic acids of formula (II), and salts, prodrugs and prodrug salts thereof:



25 where:

X is H (to form NH_2) or an amino-protecting group;

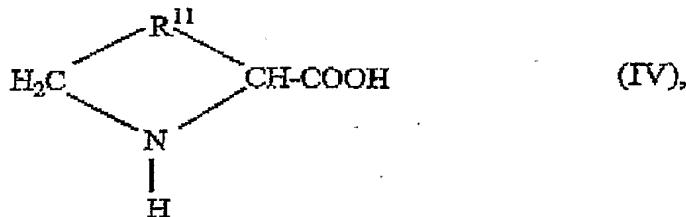
30 aa^1 is an amino acid having a side chain which is C_1-C_5 alkyl substituted by one or two moieties selected from fluorophenyl, cyclohexyl and fluorocyclohexyl;

aa^2 is an imino acid having from 4 to 6 ring members;

R^1 is a group of the formula $-(CH_2)_s-Z$, where s is 2, 3 or 4 and Z is $-\text{OH}$, $-\text{OMe}$, $-\text{OEt}$ or halogen (F, Cl, Br or I).

11. A compound of claim 9 or claim 10 wherein aa^1 is selected from 4-F-Phe, 4-F-Dpa, 4-F-Dcha
5 and 4-F-Cha, e.g. is (R)-4-F-Phe or (R)-di(4-F)-Dpa.

12. A compound of claim 11 wherein aa^2 is a residue of an imino acid of formula (IV)



10 where R^{11} is $-\text{CH}_2-$, $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{S}-\text{CH}_2-$, $-\text{S}-\text{C}(\text{CH}_3)_2-$ or $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, which group,
when the ring is 5- or 6- membered, is optionally substituted at one or more $-\text{CH}_2-$ groups by from 1
to 3 $\text{C}_1\text{-C}_3$ alkyl groups, and optionally aa^2 is an (S)-proline residue, e.g. $aa^1\text{-}aa^2$ is (R)-Phe-(S)-Pro.

15 13. A compound of any of claims 9 to 12 wherein aa^1 is of (R)-configuration and/or aa^2 is of
(S)-configuration and/or the fragment $-\text{NH}-\text{CH}(R^1)-\text{B}(\text{OH})_2$ is of (R)-configuration.

14. A compound of any of claims 9 to 13 wherein R^1 is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl, e.g. is 3-methoxypropyl.

20 15. A compound of any of claims 9 to 14 where X is $R^6-(\text{CH}_2)_p-\text{C}(\text{O})-$, $R^6-(\text{CH}_2)_p-\text{S}(\text{O})_2-$, R^6-
($\text{CH}_2)_p-\text{NH}-\text{C}(\text{O})-$ or $R^6-(\text{CH}_2)_p-\text{O}-\text{C}(\text{O})-$ wherein p is 0, 1, 2, 3, 4, 5 or 6 and R^6 is H or a 5 to 13-
membered cyclic group optionally substituted by one or more (e.g. 1, 2, 3, 4 or 5) halogens (e.g. F),
for example at least at the 4-position, and/or by 1, 2 or 3 substituents selected from amino, nitro,
hydroxy, a $\text{C}_5\text{-C}_6$ cyclic group, $\text{C}_1\text{-C}_4$ alkyl and $\text{C}_1\text{-C}_4$ alkyl containing, and/or linked to the cyclic
25 group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a
substituent selected from halogen, amino, nitro, hydroxy and a $\text{C}_5\text{-C}_6$ cyclic group, and optionally
said 5 to 13-membered cyclic group is aromatic or heteroaromatic, e.g. is phenyl or a 6-membered
heteroaromatic group, for example X is benzyloxycarbonyl.

30 16. A compound of claim 9 or claim 15 wherein the boronic acid is of formula (VIII):

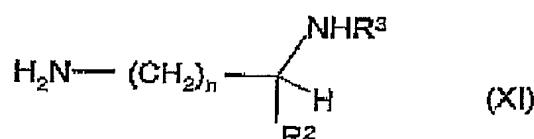
X-(R)-4-F-Phe-(S)-Pro-Mpg-B(OH)₂ (VIII).

17. A compound of any preceding claim which is in the form of a base addition salt of the boronic acid.

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18. A compound of claim 17 which comprises a salt of the peptide boronic acid with an alkali metal or a strongly basic organic nitrogen-containing compound, and optionally wherein the strongly basic organic nitrogen-containing compound is a guanidine, a guanidine analogue or an amine, e.g. comprises a salt of the boronic acid with an alkali metal, an aminosugar, a guanidine, an amine of

10 formula (XI):



where n is from 1 to 6, R^2 is H , carboxylate or derivatised carboxylate, R^3 is H , $\text{C}_1\text{-C}_4$ alkyl or a residue of a natural or unnatural amino acid, e.g. a salt with lysine, arginine or a glucamine.

15 19. A compound of claim 17 which comprises a salt of the boronic acid with a metal.

20. A compound of claim 19 wherein the metal comprises an alkali metal salt, e.g. sodium or lithium.

20 21. A compound of any of claims 17 to 20 which comprises boronate ions derived from the peptide boronic acid and has a stoichiometry consistent with the boronate ions carrying a single negative charge.

22. A pharmaceutical formulation comprising a compound of any of claims 1 to 21.

25

23. A pharmaceutical formulation of claim 22 which is adapted for intravenous administration or for subcutaneous administration, e.g. comprises the compound in the form of a finely divided solid for reconstitution as a solution ready for administration.

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24. A pharmaceutical formulation of claim 22 which is adapted for oral administration, e.g. is a tablet capsule or is a particulate formulation in a sachet.

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25. The use of a compound of claims 1 to 21 for the manufacture of a parenteral medicament for treating thrombosis, e.g. an acute coronary syndrome (for example acute myocardial infarction), a venous thromboembolic event (for example deep vein thrombosis or pulmonary embolism), for

5 preventing thrombosis in a haemodialysis circuit of a patient, for preventing a cardiovascular event in a patient with end stage renal disease, for preventing venous thromboembolic events in a patient receiving chemotherapy through an indwelling catheter, for preventing thrombosis during a coronary artery bypass graft operation, or for preventing thromboembolic events in a patient undergoing a lower limb arterial reconstructive procedure.

10 26. A parenteral pharmaceutical formulation comprising a combination of (i) a compound as defined in any of claims 1 to 21 and (ii) a further pharmaceutically active agent, for example another cardiovascular treatment agent, e.g. a lipid-lowering drug, a fibrate, niacin, a statin, a CETP inhibitor, a bile acid sequestrant, an anti-oxidant, a IIb/IIIa antagonist, an aldosterone inhibitor, an A2 antagonist, an A3 agonist, a beta-blocker, acetylsalicylic acid, a loop diuretic, an ace inhibitor, an antithrombotic agent with a different mechanism of action, an antiplatelet agent, a thromboxane receptor and/or synthetase inhibitor, a fibrinogen receptor antagonist, a prostacyclin mimetic, a phosphodiesterase inhibitor, an ADP-receptor ($P_2 T$) antagonist, a thrombolytic, a cardioprotectant or a COX-2 inhibitor.

15 27. A medicament comprising a salt, sugar ester or other soluble derivative of a boronic acid which is a selective thrombin inhibitor and has a neutral aminoboronic acid residue capable of binding to the thrombin S1 subsite linked to a hydrophobic moiety capable of binding to the thrombin S2 and S3 subsites, the hydrophobic moiety comprising a fluorinated aromatic ring in its S3-binding part and the salt comprising a cation having a valency n and having an observed stoichiometry consistent with a notional stoichiometry (boronic acid:cation) of $n:1$.

